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THE EFFECTS OF SOME OVULATION INHIBITORS ON THE DIFFERENT PLASMA LIPID FRACTIONS

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bst act A comparison has been made of the effects of ree different combined oral contraceptives (Ovulen novlar and Volidan) on the plasma levels of cholesterol glycerides, and total and individual phospholipids The ults indicate a difference in action between Anovlar on e one hand and of Ovulen and Volidan on the other

In three oophorectomized women the effects of one the estrogenic and two of the progestogenic com nents of the combined preparations were studied sepa tely The drugs were given singly or in combinations d it was found that the two progestogens produced dif rent effects as judged from the parameters studied orethisterone acetate influenced the plasma lipids in a re-tion opposite to that induced by the estrogen

These findings suggest that the overall effects on the asma lip ds induced by the various combined oral con aceptives reflect the competitional effects of the estro- ni and progestogenic components

he metabolic effects of long term usage of oral ontraceptives are of considerable interest It has een demonstrated (2 3 8 15) that these drugs ay influence the plasma lipids In an earlier udy (3) it was shown that this influence may be lated to the type of preparation

The purpose of the present study was to sub tantiate our preliminary results and to attempt n identification of the respective roles played y the different components constituting the oral ontraceptives in bringing about these changes in lasma lipids The present paper reports the re ults of analyses of cholesterol triglycerides and otal and individual phospholipids in plasma be ore and after treatment with three different ontraceptives two types of synthetic progestagens nd a synthetic estrogen preparation

MATERIAL AND METHODS

wo groups of women were stud ed (series A and B) ries A compr sed 24 healthy subjects between 23 and 7 years of age who rece ved different combined oral ontraceptives Fast ng blood samples were drawn on the 7th–8th and 22nd–23rd days of the cycle immediately preceding the first treatment period On the 5th day of the following cycle the treatment was started The tablets were given for three weeks with one tablet free week be tween each treatment period Further fasting blood samples were drawn on the first tablet day of the first, third sixth and twelfth treatment periods

Three different oral contraceptives were used, Anovlar® Ovulen® and Volidan® The composition of the prepara tions used is given in Table I Nine women received Anovlar eight completed the whole experimental period and one subject finished after the first three tablet pe riods Of the nine women taking Ovulen five subjects completed all 12 tablet periods and four patients finished after six periods In the first group one sample after the first period was lost Of the six women receiving Volidan four subjects completed the whole experiment and two patients finished after the first six periods The small size of the series does not permit any conclusion to be drawn about a possible relationship between the numbers who failed to complete treatment with the various prep- arations and their side effects

Series B comprised three women aged 43 48 and 51 years who had been oophorectomized more than two years previously A fasting blood sample was drawn on two occasions before the treatment Two women received 10 mg of Linoral® (ethinylestradiol) daily during 210 days 10 mg of Linoral and 5 m of Primolut Nor® (norethisterone acetate) for another 110 days and 10 m of Linoral and 4 mg of Niagestin® for an additional period of 21 days Fa ting blood samples were drawn at 1–3 weeks intervals The third woman received 5 m of Niagestin (megestrol acetate) for 4_ days and thereafter 4 mg of Primolut Nor for 117 days A week later she received 5 mg of Primolut Nor for ten days Blood samples were drawn every third week during treatment and one two and three weeks after discontinuing medica tion.

The plasma levels of cholesterol triglycerides and total and ind vidual phospholipids were determined as described previously (12 13)

Statistical treatment was carr ed out accordin to Sne decor (10) The in tial plasma l pid values were taken as the mean of the two values obta ned before treatment

In each case the deviations from these initial values were calculated and the possible signifi ance of the aver age deviations were tested

Table I *Preparations used in the present study*

Commercial name of preparation	Estrogenic compound	Progestagenic compound
Anovlar	Ethinylestradiol 0 05 mg	Norethisterone acetate 4 mg
Linoral	Ethinylestradiol 0 01 mg	—
Niagestin	—	Megestrol acetate 5 and 4 mg
Ovulen	Mestranol 0 1 mg	Ethynodiol diacetate 1 mg
Primolut Nor	—	Norethisterone acetate 5 and 4 mg
Volidan	Ethinylestradiol 0 05 mg	Megestrol acetate 4 mg

RESULTS

The effects of combined oral contraceptives (Ovulen Anovlar and Volidan)

Plasma cholesterol was significantly influenced only by Anovlar After one tablet period the cholesterol level had decreased in all cases After three and six cycles an average decrease was still noticeable but only at a 5% significance level After 12 periods the mean value had returned to the initial level (Fig 1)

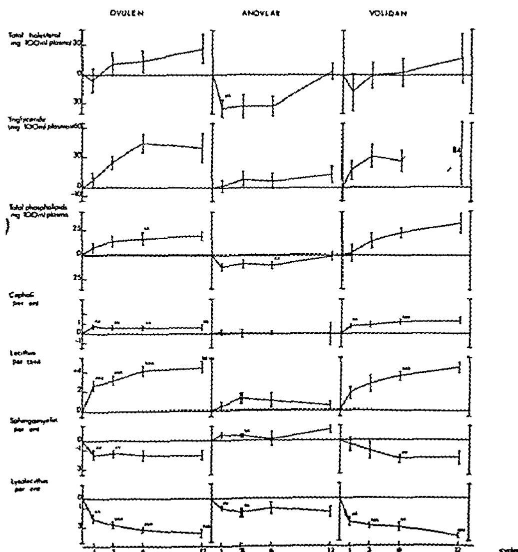


Fig 1 Changes in plasma lipid levels during treatment with Ovulen Anovlar and Volidan The bars mark the SEM for the differences between pre-treatment and treatment values The differences are considered significant at the 1% and 1‰ levels and are marked xx and xxx, respectively

Table asm i ipids uring tr n n nt ith An vla u en d ol 1

Statistical evaluation of the differences between effects obtained

	Anovlar					Ovulen					Volidan					Significance levels (*p*) for comparison between the different drugs		
	Month of treatment	M	*d*	S E M	*n*	Month of treatment	M	*d*	S E M	*n*	Month of treatment	M	*d*	S E M	*n*	Anovlar–Ovulen	Anovlar–Volidan	Ovulen–Volidan
Cholesterol (mg/100 ml)	0	238 0			9	0	211			9	0	222			6			
	1	04 8	−33 2	8 3	9	1	206	−5 6	10 46	8	1	205	−18	19 6	6	NS	NS	NS
	3	206	−32 1	11 9	9	3	223	11 5	12 1	9	3	222	−0 7	13 9	6	NS	NS	NS
	6	204 5	−32 5	10 0	8	6	226	14 5	12 82	9	6	225	2 8	14 9	6	NS	NS	NS
	12	240 5	3 5	9 1	8	12	219	26 4	12 16	5	12	239	17 5	26 0	4	NS	NS	NS
Triglycerides (mg/100 ml)	0	64 9			9	0	74			9	0	74			6			
	1	63 7	−1 2	6 1	9	1	81	7 1	7 95	8	1	92	17 8	9 5	6	NS	NS	NS
	3	73 6	8 7	7 5	9	3	100	6 7	6 33	9	3	107	32	12 2	6	NS	NS	NS
	6	73 0	5 6	7 1	8	6	121	47 8	7 72	9	6	110	36	11 3	6	<0 001	NS	NS
	12	80 3	12 9	8 5	8	12	121	40 2	15 58	5	12	165	84	31 0	4	NS	NS	NS
Total phospholipids (mg/100 ml)	0	101 9			9	0	89			9	0	99			6			
	1	89 3	−12 6	3 6	9	1	97	8 1	4 74	8	1	100	1 5	8 5	6	<0 01	NS	NS
	3	92 7	−9 2	4 2	9	3	104	14 8	4 92	9	3	114	15	7 9	6	<0 01	NS	NS
	6	91 9	−10 3	2 5	8	6	107	18 3	5 39	9	6	112	13	4 8	6	<0 001	<0 001	NS
	12	98 4	−1 5	3 0	8	12	103	21 0	4 73	5	12	121	23 3	9 9	4	<0 01	NS	NS
Lysolecithin (per cent of total phospholipids)	0	7 1			9	0	6 8			9	0	6 9			6			
	1	6 0	−1 1	0 23	9	1	4 7	−2 0	0 40	8	1	4 4	−2 4	0 35	6	NS	<0 01	NS
	3	5 6	−1 5	0 39	9	3	4 1	−2 6	0 31	9	3	4 0	−2 9	0 22	6	NS	<0 01	NS
	6	6 2	−1 0	0 67	8	6	3 5	−3 2	0 27	9	6	3 8	−3 0	0 45	6	<0 01	NS	NS
	12	5 7	−1 5	0 47	8	12	3 6	−3 6	0 42	5	12	2 9	−4 1	0 24	4	<0 01	<0 001	NS
Sphingomyelin (per cent of total phospholipids)	0	21 6			9	0	22			9	0	22			6			
	1	22 2	0 7	0 26	9	1	20 9	1 6	0 39	8	1	22	−0 5	0 72	6	<0 001	NS	NS
	3	22 1	0 5	0 10	9	3	20 8	1 3	0 26	9	3	21 2	−1 2	0 93	6	<0 001	NS	NS
	6	21 5	0 14	0 88	8	6	20 4	−1 6	0 51	9	6	20 5	−1 9	0 32	6	NS	NS	NS
	12	22 5	1 1	0 42	8	12	20 4	−1 6	0 51	5	12	20 6	−1 9	0 65	4	<0 01	<0 01	NS
Lecithin (per cent of total phospholipid)	0	68 2			9	0	68			9	0	67 8			6			
	1	68 7	0 5	0 45	9	1	70 6	2 7	0 46	8	1	69 6	1 9	0 76	6	<0 01	NS	NS
	3	69 5	1 3	0 47	9	3	71 3	3 1	0 39	9	3	70 7	2 9	0 90	6	0 01	NS	NS
	6	69 3	1 1	0 76	8	6	72 2	4 1	0 54	9	6	71 4	3 6	0 48	6	0 01	NS	NS
	12	68 8	0 6	0 41	8	12	72 0	4 4	0 69	5	12	72 0	4 5	0 55	4	0 001	<0 001	NS
Cephalin (per cent of total phospholipids)	0	3 0			9	0	3 0			9	0	3 2			6			
	1	3 1	0 07	0 15	9	1	3 8	0 9	0 17	8	1	4 1	0 8	0 06	6	<0 01	<0 001	NS
	3	2 9	−0 14	0 37	9	3	3 8	0 8	0 16	9	3	4 2	0 9	0 25	6	NS	NS	NS
	6	3 0	0 01	0 09	8	6	3 8	0 8	0 18	9	6	4 4	1 2	0 14	6	0 01	0 001	NS
	12	3 0	−0 05	1 23	8	12	4 0	0 8	0 17	5	12	4 6	1 3	0 43	4	NS	NS	NS

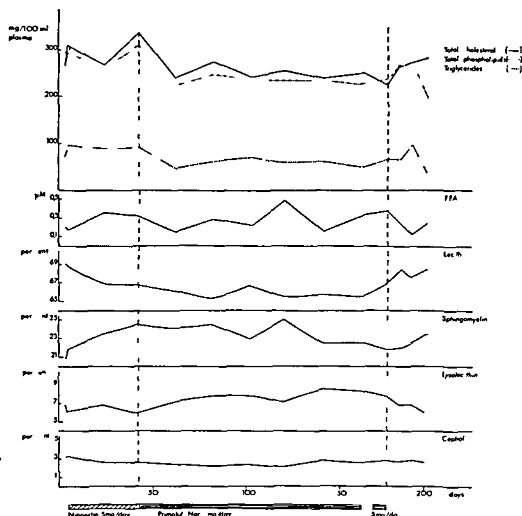


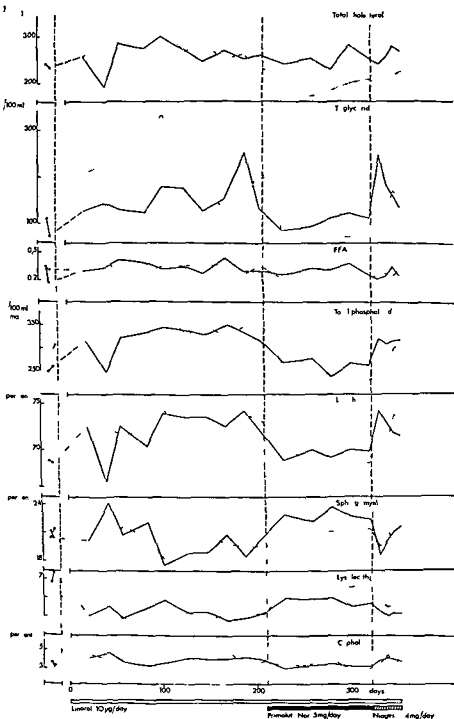
Fig Plasma lipid levels in one oophorectomized woman during treatment with Niagestin and Primolut Nor

Triglycerides were markedly and significantly increased by Ovulen and Volidan A similar tendency was observed during Anovlar treatment but the changes were less pronounced (Fig 1)

The *total phospholipid* level was possibly influenced by all preparations Ovulen and Volidan administration successively increased the level (for significance levels see Fig 1) Anovlar on the contrary lowered the level the average concentration was lower after one three and six tablet periods but after 12 periods the total phospholipid concentration had returned to the initial level (Fig 1)

Individual phospholipids The percentage of lecithin and cephalin increased during the administration of Ovulen and Volidan and the percentage of lysolecithin and sphingomyelin decreased Anovlar caused a similar but much less pronounced effect on lecithin and lysolecithin It had no effect on cephalin and caused a slight increase in the percentage of sphingomyelin The effects on the relative concentration of lysolecithin were noticeable after one month of treatment (Fig 1)

As Volidan and Ovulen seemed to have similar and more pronounced effects on the plasma lipids than Anovlar a statistical evaluation was made of possible differences between these drugs There were no significant differences between Ovulen and Volidan but there were several highly significant differences between these two drugs and Anovlar (Table II) The most pronounced differences were in their influence on the total phospholipid level and on the composition of the phospholipid fraction



3 Plasma lipid levels in two oophorectomized women ring treatment with Linoral alone or in combination th Primolut Nor or Niagestin

he effects of Linoral Primolut Nor nd Niagestin

he effects of Niagestin or Primolut Nor ad inistered alone or in combination with Linoral are illustrated in Figs 2 and 3 In the oophorectomized patient given Niagestin for 42 days (Fig 2) there seemed to be a decrease in the concentration of lecithin and an increase in the concentra

tion of sphingomyelin The other lipids were seemingly unchanged When Niagestin was exchanged for Primolut Nor there was a decrease in the levels of total cholesterol total phospholipids and triglycerides The percentage of lecithin was further decreased by Primolut Nor The percentage of lysolecithin was consistently increased by Primolut Nor After the trial period all the lipids measured returned to pre treatment levels

In the two other oophorectomized women (Fig 3) who were treated with Linoral the estrogen had caused an increase in the total phospholipid level while the cholesterol level was essentially unchanged Among the individual phospholipids the percentage of lysolecithin was markedly decreased The addition of Primolut Nor caused a decrease in the total phospholipid level and an increase in the percentage of lysolecithin The triglyceride level which had been essentially unchanged during the first three months of estrogen administration later tended to increase The addition of Primolut Nor appeared to cause a decrease in the triglyceride level After 110 days of treatment with Primolut Nor in combination with Linoral the first drug was replaced by Niagestin The lipid pattern then returned to the state before Primolut Nor treatment was started

DISCUSSION

Female sex hormones influence the metabolism of lipids and the concentration of lipids in plasma (4 6) The administration of estrogen or progestagen to oophorectomized women showed that these hormones have different effects on plasma lipids (11) The effects of oral contraceptives which include both estrogens and progestagens on plasma lipids have been repeatedly studied with somewhat contradictionary results (2 3 8 15) The relationship between female sex hormones and atherosclerosis has been discussed (1 7 9 14) It has been suggested that the influence of these sex hormones on lipid metabolism might be of importance in this connection

The present study confirms the findings reported in our preliminary report (3) that combined oral contraceptives may exert a number of effects on the plasma lipids and that the composite lipid pattern is an expression of complex interactions between estrogenic and progestogenic components

In an attempt to elucidate these effects we have undertaken a series of experiments in three oophorectomized women These patients were chosen to avoid the actions of endogenous ovarian hormones From the effects of Linoral and Primolut Nor given singly or in combination it is apparent that the estrogen directly or indirectly has an influence on a number of plasma lipids and that the progestagen produces opposite effects (Fig 3) The effects of Primolut Nor were also evident in the oophorectomized woman who received this drug without simultaneous administration of Linoral In this patient who received no estrogen the two synthetic progestagens used (megastrol acetate and norethisterone acetate) produced different effects on several plasma lipids The differences were still obvious when these progestagens were given in combination with Linoral (Fig 3) Apparently a member of the 17-α hydroxyprogesterone series (Niagestin) may have different effects from one of the 19 nortestosterone derivatives (Primolut Nor)

As an explanation for the different effects of Ovulen and Volidan on one hand and Anovlar on the other we suggest a relation to the type and amount of estrogen and progestagen Ovulen and Anovlar both contain 19 nortestosterone compounds and it might be expected that they would have similar actions provided that the differences in molecular structure were without metabolic impact Such similarity was not observed and it seems probable that the differences are caused by the different type and amount of estrogen (Table I) Anovlar and Volidan differ with respect to the progestogenic component but the estrogenic component is the same in both preparations The overall effects on the plasma lipids induced by the various combined oral contraceptives thus seem to reflect the competitional effects of the respective estrogenic and progestogenic components Volidan and Ovulen appear to have dominantly estrogenic activity Anovlar on the other hand seems to have a weak estrogenic effect on some plasma lipids and a marked progestogenic action on others It is interesting to note that an analogous situation exists in the influence of these preparations on the vaginal mucosa cells Thus Jackson (5) demonstrated that Volidan and Ovulen produces an obvious estrogenic effect and Anovlar a markedly progestogenic smear

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COUNTING OF CELLS IN URINE

The Variability of Haemocytometer Counts

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Counting of blood cells in urine is most ac y performed by means of a haemocytometer The of the method are related to technique observer ; and uneven distribution of cells in the haemocytometer

Leucocytes and non squamous epithelial cells in urine ien present in small numbers have been shown to be in the haemocytometer approximately accord to a Poisson distribution When leucocytes are present large numbers mucus and clumping of cells may with the random distribution

In quantitative estimation of cells in urine the com practice is to count the cells on a certain number f squares and to express the results as the number of lls per unit volume of urine (mm ml) or by the ex retion rate (number of cells excreted per minute hour 2 or 24 hours) The standard error of this estimate ex by the confidence interval must be based on the er of cells counted and not on this number mul iplied by a factor

It is assumed that the simplest and most convenient nethod of quantitative estimation of cells in urine is to ount the cells in 1 mm of uncentrifuged urine When he number of cells per unit volume is low the con idence limits for the true number of cells per volume in a Poisson distribution may be obtained from tatistical tables When the number of cells is larger than 40 per mm an estimate of the standard error of the count of a single sample cannot be obtained since he d stribution may not be random In routine work in formation of sufficient accuracy may be ach eved by xamination of a convenient volume containing 50–100 the number of cells per mm being calculated on he basis of this count

ng of cells in urine by means of a haemo- er was first performed by Hottinger (13) Posner (26) If lysis of cells in hypotonic or lkaline urines is disregarded the errors of this ethod are related to the accuracy of the haemo- the sampling variation the technique to fill the haemocytometer and to observational errors Given a perfect technique it has been assumed that the red cells in urine are distributed in the haemocytometer according to a Poisson distribution (28) The present study is an account of the errors and variations of haemo cytometer counts of leucocytes and non squamous epithelial cells in urine

MATERIAL AND METHODS

Seventeen urines which had been shown by routine microscopy to contain easily identifiable cells in normal or pathologically increased numbers, were selected for further study The specific gravities ranged from 1 016 to 1 0_5 and pH from 5 7 to 6 8

All counts were made by the author using the same chamber of a Fuchs-Rosenthal haemocytometer Repeated counts were made of samples from each urine using the same coverglass assembled with the haemocytometer in the same way with the same border in the same direc tion and with the same side downwards Glass surfaces wh ch would come in contact with the urine were not touched by hand Correct symmetrical positioning of the coverglass was achieved by small markings on the blocks, and symmetrical interference patterns (Newton s rings) were aimed at. If the coverglass was broken the series was discarded and the counts repeated with a new cover glass

Samples of 50 ml from each of the urines were transferred to Erlenmeyer flasks (_00 ml) and shaken for one minute Specimens were transferred to the haemocytometer with Pasteur pipettes held at an angle of about 45 Care was taken to avo d too rapid fillin_ bubbles and over flow but when this occurred the specimen was im mediately discarded As soon as the chamber had been filled it was placed on the stage of a microscope for three min before counting, so that streaming in the fluid could cease and the cells settle on the bottom of the chamber

The high dry magnification (objecti e 40/0 65) was used in counting Cells touching the left hand or and upper lines of a square were counted as being in that

Table I *Distribution of cells on 128 1 × 1 mm squares of the haemocytometer obtained in eight chamber fillings (urines no I–XVI)*

Urine no	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI
No of cells per square (x)	Number of squares having specified number of cells															
0	94	92	83	74	50	45	45	11	1							
1	28	28	37	43	52	46	34	39	10	5	3	3				
2	5	7	7	9	21	30	28	27	25	7	6	6	3			
3		1	1	1	3	6	17	23	25	23	19	17	3			
4	1			1	1	1	3	19	32	28	20	19	7		2	
5					1			5	16	29	20	13	15			
6							1	3	8	19	16	21	19	3	2	
7									9	6	11	18	22	2	2	
8								1		4	9	7	21	7	2	
9									1	4	12	6	13	6	14	
10									1	2	6	7	5	21	7	
11										1	4	3	10	10	6	
12											2	4	4	12	7	2
13												2	3	15	10	2
14														9	17	1
15													1	16	12	3
16														6	10	3
17												1	2	7	9	8
18														4	11	7
19												1		6	3	9
20														2	4	8
21														1	1	11
22															4	12
23														1	1	2
24															1	6
25																13
26															2	5
27															1	3
28																9
29																7
30																3
31–39																14
Σ x	4	45	54	68	112	128	159	289	473	600	733	782	967	1652	1827	2997

square those touching the lower or/and right hand were considered to be outside the square No count was rejected because of irregular distribution in the chamber

Counts included both leucocytes and non squamous epithelial cells since it is sometimes difficult to distinguish between these two types of cells in unstained specimens

A chamber filling represented a volume of 3 2 mm^3 over a field area of 4 × 4 mm This area consisted of 16

Table II *Distribution of cells on 128 1 × 1 mm squares obtained in eight chamber fillings (urines no I III VI IX)*

Fitted Poisson distributions

Urine no		Number of squares having specified number of cells: 0	1	2	3	4	5	6	7	8	9	10	Mean	χ^2	d f	Percentage points
I	Observed	94	28	5	0	1										
	Expected	92 16	30 23	*4 95*	*0 54*	*0 05*							0 3281	0 2 9	2	$P_{10}<\chi^2<P$[illegible]
III	Observed	83	37	7	1	0										
	Expected	83 94	35 4	*7 48*	*1 05*	*0 11*							0 4219	0 128	2	P[illegible]$<\chi^2<P_{10}$
VI	Observed	45	46	30	6	1	0	0								
	Expected	47 09	47 09	23 54	*7 85*	*1 96*	*0 39*	*0 07*					1 0000	2 938	3	P[illegible]$<\chi^2<P$[illegible]
IX	Observed	1	10	25	25	32	16	8	9	0	1	1				
	Expected	*3 18*	*11 75*	21 72	6 75	24 71	18 26	11 25	*5 94*	*2 74*	*1 13*	*0 37*	3 6953	5 055	6	P[illegible]$<\chi^2<P$[illegible]

able III *Mean number of cells and variance on 128 1×1 mm squares obtained in eight chamber llings from each of the urines no I–XVI*

D f = 127 95 confidence limits 0 769 < χ^2/d f < 1 26)

ine no	Mean x	Variance s^2	$s^2/\bar{x}$
	0 3781	0.3944	1 2051
I	0 3516	0 3872	1 1013
II	0 4219	0 4033	0 9559
V	0 5313	0 5344	1 0058
	0 8750	0 8346	0 9538
I	1 0000	0 8504	0 8504
II	1 2422	1 4606	1 1758
/III	2 2578	2 3818	1 0549
	3 6953	3 2214	0 8718
	4 6875	3 7756	0 8055
I	5 7266	6 7342	1 1760
II	6 1094	9 6887	1 5859
III	7 5547	7 6820	1 0169
IV	12 9063	12 1801	0 9437
V	14 2734	19 7908	1 3866
	23 4141	33 5988	1 4350

1 1 mm squares each of which was divided into 16 × 0.25 mm squares

From each of the urines no I–XVI eight chamber fillings were examined and the number of cells on each 1×1 mm square" recorded From urine no XVII hich contained a high number of cells six chamber lin s were examined and the number of cells per 0.25×0 5 mm square recorded

The distribution of cells in a haemocytometer is assed to follow a Poisson series The chief characteristic f the Poisson distribution is that the variance (s^2) is qual to the mean ($\bar{x}$) The ratio $s^2/\bar{x} = 1$ will follow a f distribution with the degrees of freedom (d f) de uned by the number of observations The 95 con dence interval of the distribution is obtained from ables (7)

The variability of random samples was studied by of χ^2 tests

Statistical calculations were performed by L Giezendan Kleppestø Norway

RESULTS

ne observed distribution of cell counts in 128 1×1 mm squares" obtained by eight chamber illings of each of the urines no I–XVI is shown n Table I

The observed distributions of counts for urines o I III VI and IX were very close to the ieoretical Poisson distributions and in no case a χ test indicate significant difference (Table)

The mean number of cells on 128 1×1 mm squares" the variance and the calculated ratios

Table IV *Variability of number of cells in eight random samples (3 2 mm³) from each of sixteen urines*

(D f = 7 Percentage point of χ^2 P_{95} = 14 07)

Urine no	Range of counts	Average no of cells in 8 samples ($\bar{x}$)	$\Sigma(x-\bar{x})^2$	χ^2
I	2– 7	5 25	23 50	4 48
II	3– 10	5 63	39 87	7 08
III	4– 8	6 75	13 50	00
IV	3– 14	8 50	84 00	9 88
V	10– 23	14 00	106 00	7 57
VI	12– 22	16 00	84 00	5 25
VII	13– 28	19 88	18_ 87	9 20
VIII	20– 46	36 13	426 87	11 81
IX	50– 66	59 13	2 4 87	4 31
X	6?– 86	75 00	606 00	8 08
XI	73–108	91 63	671 87	7 33
XII	86–110	97 75	387 50	3 96
XIII	94–154	120 88	? 300 87	19 03
XIV	178– ?3	_06 50	1 662 00	8 05
XV	184–258	228 38	3 977 87	17 42
XVI	_96–491	374 63	28 659 87	76 50

$s^2/\bar{x}$ for each of the urines no I–XVI are shown in Table III Comparison of the ratios with the 95% confidence interval of the χ^2/d f distribution revealed that in most cases the ratios fell within the 95% confidence limits of the χ^2/d f distribution (0 769 < $s^2/\bar{x}$ < 1 26 d f = 127) and might therefore fit Poisson distributions The cell distributions in three urines (no XII XV XVI) will not fit Poisson distributions as the ratios were beyond the range of the 95% confidence interval of the χ^2/d f distribution

The variability in cell counts in eight samples from each of urine specimens no I–XVI was examined by the χ^2 test Table IV gives the range of counts in eight samples the average number of cells per sample and the calculated χ^2 With 7 d f the percentage point of χ^2 is P_{95} = 14 07 Larger values of χ^2 indicate that the distributions have a greater variance than expected for Poisson distributions In most cases the calculated χ^2 values were within the range expected for Poisson distributions while three urines with large numbers of cells (no XIII XV XVI) showed higher χ^2 values

Table V shows the distribution of cell counts for urine no XVII on 256 "0 25×0 25 mm squares in each of the six samples (chamber fillings) together with the mean number of cells per square the variances and the calculated ratio $s^2/\bar{x}$ for each chamber filling Comparison with

Table V *Analysis of the distribution of cells on 256 0 25 × 0 25 mm squares in six chamber fillings from urine no XVII*

(D f = 255 95 confidence interval $0\,833 < \chi^2/\mathrm{d\,f} < 1\,18$) (D f = 1535 95% confidence interval $0\,920 < \chi^2/\mathrm{d\,f} < 1\,085$)

Chamber filling no	Number of squares having specified number of cells						
	1	2	3	4	5	6	Total
No of cells (x)							
0	1				1		2
1			5	2	1	1	9
2	6	6	8	4	7	5	36
3	15	12	13	20	16	19	95
4	12	19	35	33	2	21	142
5	31	34	24	35	35	30	189
6	43	37	29	22	42	38	211
7	36	33	37	41	32	42	216
8	36	9	6	27	21	33	172
9	19	3[illegible]	22	33	22	17	145
10	12	19	8	16	2[illegible]	17	114
11	17	17	10	8	17	19	88
12	13	7	11	5	9	5	50
13	10	6	3	7	4	3	33
14	2	1	4		2	1	12
15	1	1	6	2	1	2	13
16	1	[illegible]		1			4
17	1	1		1			3
[illegible]0						1	1
3[illegible]					1		
Σx	1898	1884	1831	1755	1876	1811	11 005
	7 41	7 36	7 15	6 86	7 13	7 07	7 1647
	8 59	7 92	9 85	7 77	10 62	8 0[illegible]	8 79
[illegible]	1 16	1 08	1 38	1 13	1 49	1 13	1 23

the 95% confidence limits of the $\chi^2/\mathrm{d\,f}$ distribution revealed that the chamber fillings no 1 2 4 and 6 might fit Poisson distributions ($0\,833 < s^2/\bar{x} < 1\,18$ d f = 255) but no 3 and 5 do not In all chamber fillings the variance was greater than the mean value The probability that $s^2/\bar{x}$ in all six distributions should be above unity is $(\ \ )^6 = 1\ 64$ For the total number of observations in six samples the 95% confidence interval of the $\chi^2/\mathrm{d\,f}$ distribution based on $256 \times 6 = 1536$ observations (d f = 1535) is $0\,920 < s^2/\bar{x} < 1\,085$ The ratio $s^2/\bar{x} = 1\,23$ calculated for the sum of counts consequently revealed a highly significant deviation from the expectation of the Poisson distribution

Table VI shows the total number of cells per

Table VI *Variability of number of cells on 16 1 1 mm squares in each of six chamber fillings from urine no XVII*

(D f = 15 Percentage point of χ^2 P [illegible]5 00)

Chamber filling	Cells per chamber filling	Cells per 1 1 mm square		$\Sigma(x-\bar{x})^2$	Variance s^2	χ^2	Percentage points
		Range	Mean				
1	1898	9 145	118 6 50	063	135 73	17 16	$P_{[illegible]} < \chi^2 < P_{[illegible]}$
2	1884	98–137	117 7500	8 5	188 33	23 99	$P_{[illegible]} < \chi^2 < P_{[illegible]}$
3	1831	90–148	114 4373	3484	3[illegible] 7	30 44	$P_{[illegible]} < \chi^2 < P_{[illegible]}$
4	1755	86–130	109 6875	3047	03 13	27 78	$P_{[illegible]} < \chi^2 < P$
5	18 6	91–140	114 1[illegible]50	3074	[illegible]04 93	[illegible]6 94	$P_{[illegible]} < \chi^2 < P_{[illegible]}$
6	1811	9[illegible] 135	113 1875	1886	1[illegible]5 73	16 66	$P_{[illegible]} < \chi^2 < P_{[illegible]}$

Table VII *Number of cells in the same microscopic fields counted by six observers*

	Erythro cytes	Leuco cytes	Non squamous epithelial cells	Leucocytes + non squamous epithelial cells
Techn 1	5	64	57	121
Techn 2	11	147	12	159
Techn 3	2	173	11	184
Dr 1	3	137	23	160
Dr 2	5	149	10	159
Dr 3	6	152	14	166

chamber filling for urine no XVII the range and mean values of counts per 1 × 1 mm square and variances and χ values based on the counts per 1 × 1 mm square χ values compared with the percentage points of a χ-distribution (d f = 15) revealed that the variability of counts of the chamber fillings no 1 2 and 6 are in agreement with the expectation ($s = \bar{x}$) but those of chamber fillings no 3 4 and 5 are not The sum of the six χ values (Table VI) showed highly significant deviation from the expected Poisson distribution ($\Sigma\chi$ = 142 97 d f = 6 × 15 = 90 $\chi > P_{99}$) Thus the average variance within the six chamber fillings is significantly greater than expected for a Poisson distribution

The mean number of cells per chamber filling is 1834 17 the total $\Sigma(x-\bar{x})^2 = 13\,440$ and consequently χ = 13 440/1834 17 = 7 33 (d f = 5) and $P_{80} < \chi < P_{90}$ This indicates that there is no significant difference between the means of the different chamber fillings

ERRORS OF THE METHOD

Possible errors in calibration and filling of haemocytometers

Calibration errors of haemocytometers may be due to inaccuracies of the quadratic pattern of the counting field variations of the depth of the chamber (5 20 5) non plane coverslips (4) and poor apposition of the coverslip to the slide (5)

With a well mixed specimen the density of cells per unit area of the haemocytometer has been shown to increase from the point of entrance of the fluid along the length of the chamber (15 20 23 31)

In serial investigations the influence of these possible errors can be reduced by using the same equipment and technique throughout, by correctly positioning the cover slip and by counting squares symmetrically located about the centre

Observer error in haemacytometer counts

Observer error in quantitative estimation of cells in urine is related to identification and counting of cells The influence of this error was studied in a single experiment One unstained specimen of urine was examined in a Neubauer counting chamber by three experienced laboratory technicians and three internists successively The total numbers of different types of cells were recorded None of the observers knew the results obtained by the others

Results of the experiment are given in Table VII The total numbers of white cells (leucocytes and non squamous epithelial cells combined) agreed fairly well though for one observer the count was particularly low On the other hand the observer difference in counts of specified cells was much larger than for total cell counts

DISCUSSION

The variability of haemocytometer counts of blood cells in urine has been given little attention in the literature The present investigation has shown that leucocytes and non squamous epithelial cells followed a Poisson distribution in the haemocytometer when small numbers were present but when the number was high the variance was greater than would be expected for a Poisson distribution

This observation is in contrast to the results of blood counting experiments where the standard deviations have usually been found to be smaller about 0 9 $\sqrt{m}$ while $\sqrt{m}$ is the expectation for a Poisson distribution m being the mean number per square (review of literature by Stavem (36)) These smaller standard deviations have been studied in relation to crowding of cells on small squares and to the observer s unconscious bias (4 19 22 28 32 36 37)

A factor which sometimes may distort the random distribution in the haemocytometer is the tendency of cells to move towards the lines of the grid and settle there This may be a disturbing factor if repeated counts of the same specimen are made In routine work the problem can be avoided if cells are counted as soon as the initial drift introduced by filling has ceased

In the present study variances at higher cell concentrations were greater than those expected for Poisson distributions Although the observer errors may have been partly responsible it is more likely that the properties of the cells and

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WHOLE BODY HEMATOCRIT LARGE VESSEL HEMATOCRIT RATIO IN HYPERTENSION

The Effects of Hypotensive Drugs

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Abstract Whole body hematocrit/large vessel hematocrit (WBH/LVH ratio) has been examined in 21 normotensive and 35 hypertensive subjects and found to be the same presumably as an expression of a uniform distribution of the blood volume in the two groups Moderate renal impairment the degree of the retinopathy or the height of the diastolic blood pressure do not seem to influence the ratio figures

The WBH/LVH ratio was examined during treatment with various hypotensive drugs A significant increase in the ratio was demonstrated during the influence of a single effective intravenous dose of pentolinium

Similar results were obtained during short term treatment with guanethidine alone and during long term treatment with guanethidine and hydrochlorothiazide

Treatment with hydrochlorothiazide alone caused an increase in ratio after one week and after three months but these increases were not significant

The possible causes of these alterations in the WBH/LVH ratio are discussed The advantage of employing a double isotope technique in preference to a quicker single isotope technique has been analyzed in relation to the determination of the total blood volume in patients during treatment with hypotensive drugs

In 1929 Krogh (20) and Fahræus (9) demonstrated that the hematocrit value was lower in capillary blood than in the large veins and in 1946 Gibson et al (12) found that the hematocrit value was higher in spleen than in venous blood On account of these regional differences the so called whole body hematocrit (WBH) = erythrocyte volume/erythrocyte volume + plasma volume is different from the large vessel hematocrit (LVH) Most investigators have found that WBH/LVH ratio in normal subjects is between 0 90 and 0 95 (1 4 13 16 21 22) and remains constant when there are considerable variations in the venous hematocrit (9–82 %) (4)

Previous workers (6) discussed whether there is an abnormal distribution of red cells and plasma among the various vessel areas in hypertensive subjects The WBH/LVH ratio is an indicator of this distribution and there are only a few investigations of the WBH/LVH ratio in hypertension (19) No investigations seem to have been made of the WBH/LVH ratio in hypertensive subjects during treatment with hypotensive drugs

The object of this study was

A to compare this ratio in hypertensive subjects with a control group of normotensive subjects

B to examine the ratio in hypertensive subjects during treatment with a variety of hypotensive drugs

METHOD AND MATERIAL

For the determination of red cell volume (RCV) and plasma volume (PV) a modification of a previously described double isotope dilution technique was employed (24)

Erythrocytes from approx 30 ml of the patient s blood were labelled for one hour at room temperature with 15–30 μC $Na\ CrO$ and washed twice with physiologic saline A known weighed quantity of erythrocytes suspended in saline was injected into an arm vein after a standard of this suspension had been prepared Twenty to twentyfive min later venous blood samples were withdrawn without stasis from the other arm for hematocrit and activity measurement The latter blood samples were hemolyzed by cooling below −20 C If any activity remained from previous blood volume determinations blood was withdrawn for background counting just before the injection of new activity Determinations of large vessel hematocrit were performed in duplicate in a Clay–Adams micro-centrifuge and the mean value corrected for 4% trapped plasma (3) used for calculation of RCV The

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37 Turner M E & Eadie G S The distribution of red blood cells in the haemacytometer Biometrics 13 485 1957
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Table II *Mean WBH/LVH ratio in 35 untreated hypertensive subjects in relation to renal function retinopathy and diastolic pressure*

		Ratio	S D
(*a*) Ratio and renal function			
Creatinine clearance < 70 ml/min	9 pats	0 925	0 024
Creatinine clearance > 70 ml/min	26 pats	0 925	0 033
(*b*) Ratio and retinopathy (Keith-Wagener)			
Fundus hypertonicus I+II	27 pats	0 926	0 033
Fundus hypertonicus III+IV	8 pats	0 922	0 018
(*c*) Ratio and diastolic blood pressure			
Diastolic BP 100–119 mm Hg	23 pats	0 9.7	0 045
Diastolic BP 120–140 mm Hg	12 pats	0 921	0 029

(*a*) Reduced and normal renal function
(*b*) Degree of the retinopathy fundus hypertonicus I+II and III+IV
(*c*) Diastolic B P of 100–119 and 120–140 mm Hg

Table II shows that there were but small insignificant differences in the ratio within these three groups

The hypertensive patients were subdivided into three treatment groups

Group I

Eight patients were given pentolinium intravenously in divided doses over the course of approx ½ hour (total dose 12–16 mg pentolinium bitartrate) The value for the WBH/LVH ratio determined during injection was compared with that obtained eight days later B P was measured in the supine position both before and during pentolinium injection The blood volume determinations were made when the blood pressure had stabilized at a new lower level The average blood pressure and the average WBH/LVH ratio for the eight patients are given in Table III The increase in ratio by 0 042 is significant (S E = mean error = ±0 011 p = Students test < 0 01) The average B P fall of 32 mm Hg was significant (S E = ±2 mm Hg p < 0 001) Fig 1 shows the variations in WBH/LVH ratio in the eight patients In all but one case there was a considerable increase in ratio

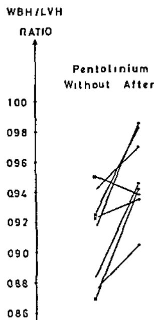


Fig 1 WBH/LVH ratio in eight hypertensive subjects without (×) and after intravenous injection of pentolinium (●)

Group II

This group comprised 17 patients who were treated with guanethidine or guanethidine combined with hydrochlorothiazide In 15 of these patients the ratio and average supine B P $\left(\frac{\text{systolic}+\text{diastolic}}{2}\right)$ were studied after 8–14 days treatment with guanethidine alone (12½–100 mg daily) and in 13 patients after 3–15 months (average 7 months) combined treatment with guanethidine (12½–50 mg daily) and hydro-

Table III *Mean WBH/LVH ratio and mean blood pressure in eight hypertensive subjects untreated and after a single intravenous dose of pentolinium*

	Untreated	Treated	Change	S E	p
Mean WBH/LVH ratio	0 910	0 952	+0 042	0 011	<0 01
Mean BP[a] mm Hg (supine)	165	133	−32	2	<0 001

Mean BP = systolic + diastolic BP/2

Table IV *Mean WBH/LVH ratio and mean blood pressure during treatment with guanethidine and guanethidine + hydrochlorothiazide*

(*a*) Before and after 8–14 days treatment with guanethidine (15 subjects) (*b*) Before and after 3–15 months treatment with guanethidine and hydrochlorothiazide (13 subjects) (*c*) After 8 14 days treatment with guanethidine and after 3–15 months treatment with guanethidine + hydrochlorothiazide (11 subjects)

	Before treatment	During treatment	change	S.E	*p*
(*a*) Mean WBH/LVH ratio	0 916	0 936	+0 020	0 006	<0 01
Mean BP mm Hg	152	135	−17	2	<0 001
(*b*) Mean WBH/LVH ratio	0 919	0 943	+0 024	0 009	<0 05
Mean BP mm Hg	156	139	−17	2	<0 001
(*c*) Mean WBH/LVH ratio	0 928	0 939	+0 011	0 009	>0 20
Mean BP mm Hg	136	134	−2	3	>0 50

chlorothiazide (25–75 mg daily) In 11 patients the ratio was studied both after treatment for 8–14 days with guanethidine alone and after 3–15 months combined treatment with guanethidine and hydrochlorothiazide The results given in Table IV show that the mean ratio in cases treated with guanethidine alone was increased by 0 020 from 0 916 to 0 936 The difference is significant (S E. = ±0 006 $p<0\,01$) The decrease in average B P 17 mm Hg is also significant (S E. = ±2 mm Hg $p<0\,001$) Fig 2 shows the variation in ratio in each of the 15 patients

From Table IV it appears that a significant increase in the ratio and a decrease in average B P are maintained by long term combined treatment with guanethidine and hydrochlorothiazide Fig 2 shows the variation in WBH/LVH ratio in each of the 13 patients A minimal decrease was found in three and an increase in ten cases In one of these cases there was a remarkable rise from 0 915 to 1 032 The average increase however was still significant when this one case was excluded

When the ratio and blood pressure during short time treatment with guanethidine are compared with the results obtained during long term combined treatment with guanethidine + hydrochlorothiazide (Table IV) there is only a non significant increase in the ratio (+0 011 S E. = ±0 009 $p>0\,2$) and a non significant drop in blood pressure (2 mm Hg S E. = ±3 mm Hg $p>0\,5$) The variation in ratio in the 11 patients

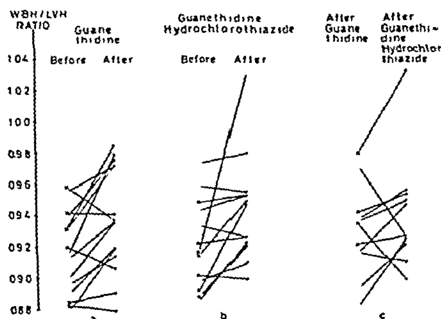


Fig 2 WBH/LVH ratio (*a*) In 15 hypertensive subjects before (×) and after 8 14 days treatment (●) with guanethidine (*b*) In 13 hypertensive subjects before ( ) and after 3–15 months treatment (●) with guanethidine and hydrochlorothiazide (*c*) In 11 hypertensive subjects after 8 14 days treatment ( ) with guanethidine and after 3 15 months treatment (●) with guanethidine and hydrochlorothiazide

Table V *Mean WBH/LVH ratio and mean blood pressure in hypertensive subjects before and after treatment with hydrochlorothia ide*

	Before treatment	After treatment	Change	S.E	*p*
A Before and after 8–14 days treatment (10 subjects)					
Mean WBH/LVH ratio	0 942	0 959	+0 017	0 012	>0 1
Mean BP mm Hg	141	121	−20	5	<0 005
B Before and after treatment in 3 months (9 subjects)					
Mean WBH/LVH ratio	0 942	0 963	+0 021	0 019	>0 3
Mean BP mm Hg	141	116	−25	4	<0 001

illustrated in Fig 2 shows no definite tendency In two cases there was a considerable decrease and in three considerable increase in WBH/LVH ratio In the remainder only minor differences were demonstrated

Group III

In ten patients the WBH/LVH ratio and average blood pressure were examined before and after 8–14 days treatment with hydrochlorothiazide

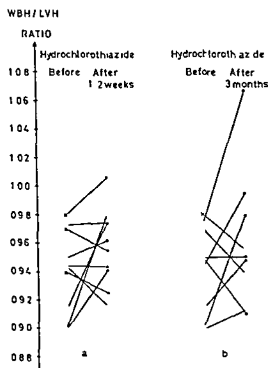


Fig 3 WBH/LVH rat o (*a*) In ten hypertensive subjects before (×) and after 8–14 days treatment (●) with hydrochlorothiazide (*b*) In nine hypertensive subjects before (×) and after three months treatment (●) with hydrochlorothiazide

alone (75 mg daily) In nine patients the examinations were repeated after three months continuous treatment with the same dose Table V shows that the initial treatment caused an increase in the ratio of 0 017 which was not significant (S.E. = ±0 012 $p > 0$ 1) On the other hand the fall in average blood pressure was significant 20 mm Hg (S.E. = ±5 mm Hg $p < 0$ 005) After treatment for three months (Table V) an increase in the ratio was again found but the difference of 0 021 was still not significant (S.E. = ±0 019 $p > 0$ 3) whereas the fall in average blood pressure of 25 mm Hg was as before significant (S.E. ±4 mm Hg $p < 0$ 001) Fig 3 shows the variation in ratio in each of the patients after short time management with hydrochlorothiazide There was an increase in five cases a decrease in three and in two cases the figures were practically unaltered Fig 3 shows also the variation in the ratio in the individual patients before and after long term treatment with hydrochlorothiazide In five cases the ratio increased in three it decreased and in one case no alteration was seen

DISCUSSION

In this study a slightly higher ratio has been found—both in hypertensive subjects and control subjects—than that reported by most of the investigators listed in Table I (4 13 16 19 21 22) The cause of this difference is not clear Some of the investigators have measured the plasma volume with Evans blue (T 1824) but this method gives the same results as ^{131}I albumin (18)

Like Jones et al (19) we have found the same ratio in both hypertensive subjects and a normotensive control group but we have not been able

to verify the larger spread in ratio found by these authors in patients with hypertension A condition for a fixed ratio is the constant percentage distribution of the blood volume among the different vascular compartments capillaries sinusoids and large vessels and a constant proportional relation between the hematocrit values in these vessel areas. Any factor which alters this relationship will also influence the WBH/LVH ratio Thus our results might indicate that the distribution of the blood volume among the different vascular compartments is the same in hypertensive and normotensive subjects Furthermore we have found in our limited material that the ratio was independent of moderate renal impairment the degree of hypertensive retinopathy and the height of the blood pressure The pretreatment ratio in group 3 was somewhat higher than in groups 1 and 2 namely 0 942 0 910 and 0 91(respectively However the difference was *not significant*

A possible increase in ratio must be a secondary to a relative reduction of the vascular compartments with a small vessel hematocrit This can be achieved by 1 a partial closing up f the capillary bed 2 by so considerable an crease in vessel diameter in a part of the capillary area that these vessels obtain a hematocrit identical or nearly identical with LVH 3 by an increase of the blood volume confined solely to vascular areas with large vessel hematocrit or 4 by a combination of two or three of these factors

In this study there was a significant increased WBH LVH ratio of 0 042 after intravenous injection of pentolinium As the total blood volume was unaltered a considerable alteration in the distribution of blood between large and small vessels must have occurred Several investigations have shown that during the influence of ganglionic blocking agents the peripheral vascular resistance is either reduced or unaltered (11 14 28) Therefore it is likely that the alteration in ratio is due to dilatation of a considerable part of the small vessels and a consequent increase in the hematocrit value in these small vessels

The WBH/LVH ratio increase with guanethidine treatment (0 020) was only about half as great as that seen with pentolinium (0 042) However it must be remembered that the reduction of mean blood pressure was far greater with pentolinium than with guanethidine (in supine position 32 mm Hg and 17 mm Hg respectively) and most of the pentolinium treated patients were unable to assume an erect position. In hypertensive patients treated with ganglionic blocking agents an accumulation of blood on the venous side of the systemic circulation is known to take place (10) Probably this also occurs during treatment with guanethidine (23) The increase in ratio during treatment with guanethidine was accompanied by an increase in the total blood volume of more than 8 per cent (25) If this increase had taken place solely in areas with larger vessel hematocrit this would account for at least part of the augmentation in ratio Our investigations do not allow us to state if this alone can explain the increase in ratio or if there has been a simultaneous dilatation of small vessels Previous investigations offer no explanation as they seem to point to the fact that the hypotensive effects of guanethidine are due to a *reduction in cardiac output more than to a reduction in peripheral resistance* (5 8 23)

Long term combined therapy with guanethidine and hydrochlorothiazide had—as appears from Table IV—almost the same effect on the ratio and blood pressure as therapy with guanethidine alone but without change in blood volume (25) During treatment with thiazides alone reduced peripheral resistance and a higher cardiac output have been demonstrated after a few weeks (7 27) In guanethidine treatment as mentioned above an unchanged or reduced peripheral resistance accompanied by reduced cardiac output is found As blood volume during combined treatment is unaltered and considering the separate action of the two drugs and their synergistic hypotensive effect it must be assumed that the increased ratio is a consequence of a dilatation in the capillary bed with a resultant reduction of the areas with small vessel hematocrit

During treatment with hydrochlorothiazide we have demonstrated a significant reduction in the average total blood volume of about 5% both after eight days and after three months treatment (15) At the same time there was a non significant increase in the ratio of 0 017 and 0 021 respectively These results do not allow us to make any statement as to the effect of hydrochlorothiazide on the distribution of the plasma and red cell in the vascular system

We have used a rather time-consuming double

isotope technique In normotensive and untreated hypertensive patients the WBH/LHV ratio is a rather constant factor In these cases it would be possible with satisfactory accuracy to determine the total blood volume by using the more simple indirect methods either on the basis of erythro cyte volume or plasma volume according to the following formulas

Total blood volume (from formulas 1 2 and 3)

$$= \frac{RCV}{WBH} = \frac{^{51}Cr\ vol}{WBH/LVH} \qquad (5)$$

Total blood volume (from 1 and 5)

$$= \frac{PV}{1 - WBH} = \frac{PV}{1 - WBH/LVH \times LVH} \qquad (6)$$

On the other hand previous investigations have shown that the ratio can be altered under certain circumstances a high ratio has been demon strated in pregnancy (21 27) whereas the ratio can be low in patients with congestive heart disease acute hemorrhage postoperative shock conditions and some cases of pheochromocytoma (2 17)

Our investigations stress the fact that the ratio is not a completely stable factor during the ef fects of hypotensive drugs as short term in fluence by pentolinium as well as prolonged treat ment with guanethidine caused significant in creases in the ratio whereas the increase during hydrochlorothiazide treatment was not significant As the blood volume during the effects of pento linium was unaltered and the WBH/LVH ratio increased by about 4% an erythrocyte method according to formula 5 would have overestimated the total blood volume by about 4% if a fixed ratio had been used Conversely a plasma method according to formula 6 would have under estimated the blood volume Neither method would have given information about the altera tions in the WBH/LVH ratio Such information could only have been obtained by a simultaneous determination of the erythrocyte and plasma volume

In the patients treated with guanethidine the ratio was increased by about 2% and therefore an indirect method according to formula 5 or 6 would have over-determined or under-determined respectively the total blood volume by about 2% By a less meticulous assessment of the alterations of the blood volume during treatment with hypotensive drugs it would be warrantable to employ either an erythrocyte method or a plasma method To obtain a true picture of the effect of hypotensive drugs on the blood volume it is necessary to employ the more time-consuming method with simultaneous determination of the erythrocyte and plasma volumes

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IG G IG A AND IG M GRANULOCYTE REACTIVE ANTINUCLEAR FACTORS IN RHEUMATOID ARTHRITIS

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Abstract Sera from 84 patients with rheumatoid arthritis were investigated for Ig Ig-G Ig A Ig M ANF reactive with nuclei of polymorphonuclear granulocytes using a two- and three layer immunofluorescent antibody technique

Granulocyte reactive ANF were found in / of the sera. Of the 56 positive sera 60 contained Ig G ANF 67 % Ig A and 67% Ig M ANF Sera containing granulocyte reactive ANF exclusively of high molecular weight (Ig A and Ig M) constitute 39% of the positive sera

Granulocyte reactive ANF were found significantly more frequently in sera from patients 61 years old or older owing to an increase of Ig G ANF No sex difference was demonstrated There was no statistically significant relationship between gran react ANF of the three human immunoglobulin classes and such clinical parameters as ESR functional capacity or concentration of haemoglobin

A strict correlation between granulocyte reactive ANF and the presence of nodules was found all patients with nodules showing a positive ANF reaction

Half the 56 positive sera reacted exclusively with nuclei of polymorphonuclear granulocytes while the other half also reacted with other human nuclei With advancing disease increasing non specificity of ANF occurred and a significantly higher proportion of sera from patients with long lasting disease also contained ANF reactive with other human nuclei *This increase in nuclear reactivity* was accompanied by a change in the immunoglobulin pattern of granulocyte-reactive ANF the Ig-G ANF being found significantly more frequently in sera from patients with disease of short duration, while Ig G ANF were present in a significantly higher proportion of sera from patients with more long lasting disease

In rheumatoid arthritis ANF reactive with polymorphonuclear granulocytes (gran react ANF) have been found in a very high proportion of sera. Thus Alexander et al (1) found gran react. ANF in 65 Hasker et al (16) in 70 and Elling et al (11) in 66% of sera from patients with rheumatoid arthritis In contrast ANF reactive with other human nuclei and with nuclei from animals have been found with a considerably lower incidence ANF in rheumatoid arthritis thus seem to exhibit a limited nuclear reactivity directed mainly against nuclei of polymorphonuclear granulocytes

The highest incidence of gran react ANF in rheumatoid arthritis is found when granulocytes altered in some ways are used as nuclear antigen possibly owing to an inaccessibility of intact granulocytes to ANF of high molecular weight (Ig A and Ig M) or to ANF of these two immunoglobulin classes preferentially reacting with altered nuclear material (10) Since the incidence of ANF in rheumatoid arthritis determined by using altered granulocytes is approximate twice that obtained by using unaltered granulocytes (11) the proportion of sera containing ANF of high molecular weight would be expected to be high Although it has been stated that ANF in rheumatoid arthritis are mainly macromolecules (2 5 17) it is difficult to use these results directly since the choice of nuclear antigen and the degree of alteration apparently play a major role in determining the incidence and immunoglobulin classes of ANF

This study was undertaken with the object of determining the incidence of ANF reactive with altered granulocytes and the immunoglobulin classes of these ANF Previous reports have indicated a relationship between gran react ANF and the severity and activity of disease (1 8) and produced some evidence of there being a certain immunoglobulin pattern of ANF related to duration of disease suggesting the existence of a primary and a secondary response (3) Attempts

were therefore made to evaluate the clinical and biological roles of gran. react ANF in rheuma toid arthritis.

MATERIAL

The material consisted of 84 patients with definite or classical rheumatoid arthritis according to the ARA cri teria, selected at random from 100 patients in the King Christian X Arthritis Sanatorium at Gråsten.

METHODS

The two-layer fluorescent antibody technique was used to determine Ig ANF and a three layer technique was adopt d to determine the immunoglobulin classes of ANF (Ig-G Ig A and Ig M)

The *nuclear substrate* was blood smears prepared from a single healthy human donor The slides were frozen within five minutes by placing the smears on dry ice After thawing, the freezing and thawing were repeated twice since previous assays had shown this procedure to provide optimal antigenicity (10) Human thyroid glands and gastric mucosa obtained at operation were used as nu lear antigen.

The *antisera* were 1 antiserum prepared in rabbits and containing anti-human-gammaglobulins reactive with human immunoglobulins as described previously (12) 2 specific antisera prepared in rabbits against human Ig-G Ig A and Ig M immunoglobulin respectively (obtained from Central Laboratories, Red Cross Amsterdam) —the immunochemical specificity of these antisera was ascertained as previously described (10) 3 antirabbit gammaglobulin prepared in sheep and shown by immunoelectrophoresis to react only with rabbit gammaglobulin (supplied and tested by B Mansa, Biophysical Depart ment, Statens Seruminstitut) Antisera 1 and 3 were con jugated with 25 mg fluorescein-isothiocyanate per g protein.

Performance of test In the two-layer technique Ig ANF were determined by using conjugated rabbit anti-human gammaglobulin, while in the three layer technique uncon jugated rabbit anti human gamma-globulin, specific for Ig-G Ig A and Ig M immunoglobulins, was applied as the second layer after serum After washing, the slides were incubated with conjugated sheep anti rabbit Ig-G gammaglobulin for 30 minutes and then, after repeated washing, mounted in buffered glycerol with cover slips.

Appropriate control slides without serum and slides with negative and positive sera were included in all ex periments. The microscope was a Leitz Zernicke with equipment for simultaneous phase-contrast and fluorescent microscopy All slides were examined in phase-contrast to ascertain that granulocytes and lymphocytes were present. Conjugates when used alone were never observed to produce any nuclear staining.

Rheumatoid factors were determined as described pre viously (6) using the sensitized sheep cell agglutination test and with the FII sensitized latex test (Hyland reagent)

NUMBER OF PATIENTS

Fig 1 Immunoglobulin pattern of granulocyte reactive ANF in rheumatoid arthritis

RESULTS

Incidence (Table I and Fig 1)

Gran. react. ANF were present in 56 of 84 sera (66 4 %) all showing a homogeneous fluorescence of nuclei of polymorphs One third of the sera exhibited a strong and bright fluorescence and the remainder showed weaker reactions (+ to + +)

Ig-G gran react ANF were found in 60 7 % Ig A gran. react. ANF in 62 4 % and Ig M gran react. ANF in 67 8 % of the 56 positive sera. This shows that sera containing gran. react ANF exclusively of high molecular weight (Ig A and Ig M) constitute 39 3 % of the positive sera

Fig 1 shows that approximately one third of the positive sera contained gran react. ANF of

Table I *Incidence and immunoglobulin pattern of granulocyte reactive ANF in 84 patients with rheuma toid arthritis*

	Gran. react. ANF			
	Ig	Ig–G	Ig–A	Ig–M
Female	43 65 66 %	26/43 60 %	26 43 60	30 43 69 %
Male	13 19 68	8/13 61	9 13 69	8/13 61 %
Female and male	56 84 66 %	34/56 60	35/56 6 %	38 56 67 %

Table II *Relationship of granulocyte reactive ANF to age of patients*

	Gran react ANF			
Age	Ig	Ig–G	Ig–A	Ig–M
21–50	17/30 56%	9/17 52	10/17 58	11/17 64%
51–60	22/34 64%	12/22 54	16/22 72%	16/2[illegible] 72%
61–	17/20 85	13/17 76	9/17 52%	11/17 64%

one immunoglobulin class one third contained gran react. ANF in two immunoglobulin classes while in one third of the sera gran. react ANF were found in all three human immunoglobulin classes

Half of the 56 positive sera reacted exclusively with nuclei of granulocytes while the other 28 sera also reacted with other human nuclei (thyroid nuclei gastric mucosa nuclei and lymphocytes) No statistically significant difference in the immunoglobulin pattern in these two groups was found

Sex (Table I)

No statistically significant difference between sexes was found with respect to the occurrence of Ig Ig G Ig A or Ig M gran react ANF

Age (Table II)

A statistically significant increase ($p = 0\ 0468$) in Ig gran react ANF was found with increasing age 85% of patients aged 61 years or older had gran react. ANF This finding was caused by an increase in the Ig-G ANF while Ig A and Ig M ANF showed no significant variation in the different groups of patients

Table IV *Relationship between granulocyte reactive ANF and erythrocyte sedimentation rate*

	Gran. react. ANF			
ESR	Ig	Ig–G	Ig–A	Ig–M
–19	7/13 53%	3/7 42	5/7 71	6/7 85%
20–39	15/23 65	10/15 66	9/15 60	10 15 66
40–59	15/22 68%	10 15 66	10 15 66	12/15 80
60–	19/26 7[illegible]%	11/19 57%	11/19 57%	10/19 5[illegible]%

Duration of disease (Table III)

In patients with disease of short duration (less than five years) a significantly (5% level) higher incidence of Ig-G gran react. ANF was found while in patients with disease of longer duration there was a significantly higher incidence of Ig A gran react. ANF Ig M ANF tend to occur more frequently in sera from patients with disease of short duration but the figures are not statistically significant at the 10% level

Five of seven patients with disease of less than one year's duration gave a positive test for gran react ANF and in only one of these sera were the gran react. ANF found to be of the Ig A immunoglobulin class

The change in the immunoglobulin pattern of gran react ANF with advancing disease was accompanied by a significant ($p = 0\ 0302$) increase in the incidence of ANF which were also reactive with other human nuclei All the positive sera from patients with duration of disease less than

Table III *Relationship of granulocyte reactive ANF to duration of disease*

Duration of disease (y)	Gran react. ANF				Gran specific ANF	Positive sera (%)
	Ig	Ig–G	Ig A	Ig–M		
<1	5/7 71%	3/5 60%	1/5 20	2/5 40%	5/7	100
1–4	17/31 54%	14/17 8	9/17 52%	14/17 82%	13/31	76
≥5	34 46 74%	17/34 50%	25/34 73	2[illegible]/34 65%	10 46	21

Table V *Relationship between granulocyte reactive ANF and stage of disease*

Stage	Gran react. ANF Ig	Ig-G	Ig-A	Ig-M
1-2	16/25 65	9/16 56	7/16 43	9/16 56
3-4	40/59 67	22/40 55	25/40 6?	26/40 65

Table VI *Relationship between functional capacity and granulocyte reactive ANF of different immunoglobulin classes*

Functional capacity	Gran react ANF Ig	Ig-G	Ig-A	Ig-M
1-2	39 60 65	23/39 58	23/39 58*	28/39 67
3-4	17/24 70	11/17 64	12/17 70	10/17 58

Table VII *Relationship between concentration of haemoglobin and granulocyte reactive ANF*

Concentration of Hb ( )	Gran react ANF Ig	Ig-G	Ig-A	Ig-M
-80	11/18 61	5/11 45	8/11 72	7/11 63
80-90	26/39 66	15 26 57	16/26 61	18/26 69
90-	18 26 69	13/18 74	10/18 55	13/18 74

Table VIII *Relationship between rheumatoid factor (latex test) and granulocyte reactive ANF*

F II sensitized latex test	Gran react. ANF Ig	Ig-G	Ig-A	Ig-M
Negative	5 17 29	3/5 60*	4/5 80	4/5 80
Positive ≥20	51/67 76	31 51 60	31/51 60	35/51 68
≤1/160	32 47 68	20 32 62	20 32 6	22/32 68
>1/160	19/20 95*	11 19 58	11 19 58*	13/19 68

Table IX *Relationship between sensitized sheep cell agglutination test and granulocyte reactive ANF*

Sheep cell agglut. test	Gran react ANF Ig	Ig-G	Ig-A	Ig M
Negative <20	15/31 48	10/15 66	10/15 66	12/15 75
Positive ≥20	40/52 76	24/40 60	24/40 60	26/40 65*
≤1/160	26/35 74	15/26 57	15/26 57	17/26 62
>1/160	14/17 82	9/14 64	9/14 64	9/14 64

one year and 3/4 of those from patients with duration of disease less than four years contained granulocyte *specific* ANF but this factor was only present in 21 % of the ANF positive sera derived from patients with duration of disease longer than five years

No statistically significant difference at 5 % level was found between the incidence of gran react ANF of any immunoglobulin class and ESR (Table IV) *stage* (Table V) *functional capacity* (Table VI) or *concentration of haemoglobin* (Table VII)

Rheumatoid nodules

A strict correlation between the presence of nodules and gran react ANF was found and all patients with nodules showed a positive reaction for ANF The patients with nodules however accounted for only 1/4 of the 56 positive sera *and the remaining 3/4 of these patients showed* no signs of nodules

Rheumatoid factors

With both the latex test and the sheep cell agglutination test no statistically significant difference in the immunoglobulin pattern of gran react. ANF was found in sera showing positive or negative reactions for rheumatoid factors The relationship found between these factors has been described previously (11)

DISCUSSION

In this study ANF reactive with polymorphonuclear granulocytes were found in /3 of sera from patients with definite or classical rheumatoid

arthritis including cases of all degrees of severity and duration of disease This factor thus occurs with the same prevalence as the rheumatoid factors determined by the sensitized sheep cell agglutination test

Only a slight difference in the frequency of gran react. ANF of the three human immunoglobulin classes Ig-G Ig A and Ig M was found However it is of importance that 22 of 56 positive sera (approximately 40 %) were found to contain gran react ANF of the Ig A and Ig M immunoglobulin classes only since gran react ANF of these two immunoglobulin classes have been shown to react preferentially with granulocytes that have been altered in some way (10) e g by repeated freezing and thawing Thus the incidence of positive sera may differ by almost 40 depending on the degree of alteration of the granulocytes It is therefore likely that the high proportion of sera containing gran react ANF of the Ig A and Ig M immunoglobulin classes may explain the low incidence of gran react ANF found by some authors using non altered granulocytes

The incidence of gran react ANF was found to be increased in some age groups and was 85% in patients 61 years or older Most of these patients showed signs of active disease and this may explain the high incidence which not necessarily is caused by a greater ability of older people to produce auto-antibodies

The Ig G gran react ANF *tend* to occur more frequently in sera from patients with disease of short duration low functional capacity and high incidence of rheumatoid factors while Ig A gran react. ANF were mostly found in sera from patients with disease of long duration poor function and low concentration of haemoglobin The Ig M gran react ANF were mostly present in sera from patients with disease of short duration ..t in contrast to patients with Ig G ANF these ..ents tend to have good functional capacity ..h concentration of haemoglobin and low ESR However there was no statistically significant relationship between gran react ANF of any immunoglobulin class and clinical parameters such as ESR functional capacity and concentration of haemoglobin

It is not known whether ANF are produced .. an active immunization If they are the pri.. response should probably be an Ig M ANF since gran react ANF of this immunoglobulin class were the first to appear in patients with juvenile rheumatoid arthritis (3) followed almost immediately by the Ig-G ANF while Ig A gran react ANF were only present in one of the children investigated In the present study five of seven patients with disease of less than one year s duration showed positive reaction for gran react ANF in one case only were the gran react ANF of the Ig A immunoglobulin class the remainder being Ig M or Ig G granulocyte *specific* ANF In the material as a whole Ig-G gran react ANF were found significantly more frequently in sera from patients with disease of short duration the Ig M gran react ANF showed a similar but not significant tendency while Ig A gran react ANF apparently develop later in disease It is uncertain whether this finding should be interpreted as evidence of the presence of a primary and secondary response but if this is so they indicate that the gran react ANF in rheumatoid arthritis is produced through an active immunization

Such a concept may be supported in part by the finding that the shift in the immunoglobulin pattern with advancing disease was followed by a significant increase in the number of sera which also react with other human nuclei since increasing non specificity is known to occur with long lasting immunization (7) The increasing non specificity may be caused by an increasing cross reactivity of gran react ANF or to the occurrence of ANF with other nuclear specificities Absorption studies with isolated nuclei of granulocytes show that such nuclei may be capable of absorbing ANF reactive with other nuclei (9) thus suggesting some cross reactivity of gran react ANF However great difference in nuclear reactivity has been reported (3 14 15) indicating the presence of several antinuclear globulins and such ANF may also account for the decrease in nuclear specificity found in sera from patients with long standing disease

Normal human serum apparently contains nuclear material which injected into rabbits gives rise to the production of antinuclear factors specifically directed against nuclei of polymorphonuclear granulocytes (4) Thus there is the possibility of gran react ANF being produced through an active immunization which only differs quantitatively from the normal In patients

with rheumatoid arthritis the nuclear material may possibly be derived from the many poly morphs which invade the inflamed joints since preliminary data from this laboratory show that gran specific ANF in some cases can be detected in the synovial fluid without any traces in the corresponding sera. That some predisposition of the patients or some alteration of the nuclear material (e g in the synovial fluid or in other inflammatory sites) apparently is necessary appears to be indicated by the lack of gran. *specific* ANF in patients with leucopenia of various etiol ogies including patients with cyclic granulocytopenia, in patients iso-immunized with human serum and in patients with a great turn-over of granulocytes e g myeloid leucosis (13) Work is in progress to show whether *any* long lasting in flammatory foci lead to the formation of gran specific ANF but so far this factor appears to occur almost exclusively in patients with rheu matoid arthritis and SLE.

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AN INTRAVENOUS GALACTOSE TOLERANCE TEST AND ITS USE IN HEPATOBILIARY DISEASES[1]

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Abstract The paper describes an intravenous galactose *tolerance test performed by a single injection of 350 mg* of galactose per kg body weight Galactose in the blood was determined by a specific galactose oxidase method

The disappearance of the galactose from the blood mainly occurs by hepatic metabolism which is rate limited The course of the elimination curve however is also influenced by extrahepatic factors The curve was found to be most easily described as a straight line in a semilogarithmic system the T ½ value for galactose being found to be 12 0 ± 2 6 min in normal adults The galactosuria was reasonably small and constant in different clinical conditions and was therefore disregarded

The T ½ value was elevated in 84 of cases of liver cirrhosis, and the elevation appeared to be a good measure of the degree of liver damage Abnormal test results were obtained in 58% of patients with acute infectious hepatitis. Among patients with an abnormal T ½ value more than three weeks after the onset of jaundice a long duration of the disease was generally seen the development of cirrhosis was demonstrated in one isolated case and one patient died In patients with cancer metastases of the liver the test result was abnormal in 50 The T ½ value was normal in 93 of cases of biliary obstruction

The galactose test was found to give valuable diagnostic and prognostic information in liver cirrhosis and in hepatitis and also to be useful in differentiating between obstructive and parenchymatous jaundice

A renewed interest for galactose tolerance tests in investigating liver function has become evident since the occurrence of serious complications with the Bromsulphalein retention test some fatal (13 29) has called for an alternative method A galactose test is known to be harmless to the patient except in the rare cases of hereditary galactosaemia

In the Scandinavian countries extensive investigations of the galactose load as a liver function test have been made by Stenstam (20) and Tygstrup (23) who have also given good reviews of earlier work in this field

It is generally agreed that the galactose tolerance test should be made by the intravenous route in order to avoid influence on the result by variations in gastrointestinal absorption

The aim of this investigation was to evaluate the clinical use of a tolerance test made by a single injection of galactose Relatively few results of this form of the galactose test have been reported earlier with regard to conditions other than cirrhosis of the liver

The test method which is a modification of those published by Colcher et al (4) and Vink (25) was intended to be comfortable for the patient and simple for the technician

METHODS

The subjects were always investigated in the morning They were kept in bed during the test, prior to which they fasted for eight hours this including moderate restrictions in water intake

350 mg of galactose per kg body weight were administered intravenously over a period not exceeding three minutes Commercially available 25 (w/v) sterile and pyrogen free solution was used It was known to contain about 7% of other reducing substances mainly glucose (5%) The solution was generally administered by means of 50-ml syringes and a scalp vein needle of 1 4 mm diameter inserted into a cubital vein The scalp vein needle was found to be particularly suitable for the purpose as it permitted comfortable and rapid changing of syringes during the injection without risk of dislocating the needle

In some cases the galactose was given as an intravenous drip infusion directly from a calibrated flask a

[1] Part of this work was first presented at the meeting of The Swedish Society of Internal Medicine November 28 1964

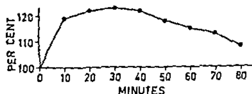


Fig 5 Blood glucose concentration during galactose tests as per cent of the fasting value Mean of 20 patients

The limit between normal and abnormal T $^1/_2$ values was defined here as the mean value for the whole normal case material +2 S D which means that T $^1/_2$ values above 17 0 min were regarded as abnormal

Reproducibility

The error of the method was determined by repeated tests in 14 patients including patients with normal and patients with abnormal results (Fig 4) The interval between the galactose loads was less than two weeks and no apparent change had occurred in the patients state of health in the meantime

The standard error of a single determination was ±1 9 min corresponding to ±13 % of the mean T $^1/_2$ value of the group

Excretion of galactose and glucose after galactose load

The urinary excretion of galactose during the tolerance test was determined in 41 of the 56 normal patients and was 7 3±3 5 % (mean± S D) of the administered dose The excretion among the 21 patients with a galactose T $^1/_2$ value of 12 0 min or below was 7 0±3 8 % Among the patients with a T $^1/_2$ value above 12 0 min the excretion percentage was 7 6±3 2

The galactosuria was also determined in connexion with 40 galactose tests in patients with cirrhosis the excretion being 7 8±5 7 % of the dose given

In 11 patients who received 700 mg of galactose per kg body weight the urinary excretion was 11 0±6 5 % of the dose

Sometimes the urine was found to have a positive reaction to glucose when tested with a Clinistix® paper strip This occurred in patients without diabetes and even in cases with no measurable galactosuria.

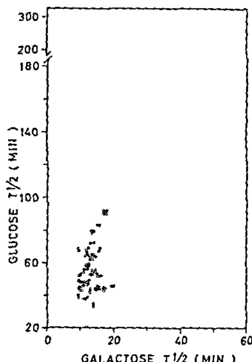


Fig 6 Comparison between intravenous galactose and glucose tolerance tests in 165 patients ● patients from the normal case material ○ other patients

Blood glucose values during galactose tolerance test

Blood glucose was determined during the galactose test in 20 of the normal patients An immediate increase of about 20 % of the fasting value was seen followed by a gradual return towards the initial level (Fig 5)

Effect of glucose on galactose elimination

In 13 patients with a normal or abnormal galactose T $^1/_2$ value the test was repeated within a few days these patients were not however identical with those from the series of duplicate determinations At the second test the galactose was administered together with an equal amount of glucose

The mean difference between the paired T $^1/_2$ values was 1 9±3 9 (S D) min the tests in which glucose was added representing the higher mean value This difference is not significant ($p > 0\ 10$)

Comparison between galactose tolerance and glucose tolerance

Intravenous tolerance tests with galactose and glucose on different days were performed in 165

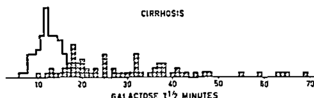


Fig 7 Galactose T ½ values for patients with cirrhosis of the liver Each shaded square represents a test result. The unfilled area shows the distribution of values in the normal case material

patients 38 of whom belonged to the normal series The others represented a wide variety of disorders both with and without affection of the liver In only four cases was diabetes the main diagnosis Fig 6 shows that there is no correlation between the two tests

Complications due to galactose tolerance tests

More than nine hundred tests have been performed here by this method without the occurrence of any systemic or local side effects of the injected galactose Up to seven tests have been made on the same patient at intervals varying between one day and several months

Liver cirrhosis

In the patients with cirrhosis of the liver the T ½ values of the 70 tests ranged between 9 5 and 69 0 min (Fig 7) with a mean value of 29 5 min The test result was abnormal in 59 cases (84 %) The mean value of 11 tests in the patients with portacaval anastomosis was 33 5 min (range 18 0–58 5 min) Two patients were examined both before and after the anastomosis was made In one of them the T ½ value had risen from 13 0 to 18 0 min two weeks after surgery in the other case the T ½ value was 18 0 min before and 30 0 min 25 days after the operation

Jaundice or ascites or a combination of both was present in connexion with 33 tests The mean T ½ value in these cases was 35 3 min (range 11 5–69 0 min) The mean value in the patients without these signs was 24 2 min (range 9 5–58 5 min) (Fig 8)

The mean T ½ value in 26 tests in the 21 patients who subsequently died was 34 8 min (range 14 0–65 0 min) compared with 24 3 min (range 9 5–48 0 min) in 32 tests in the 26 surviving patients who were kept under observation for longer than half a year (Fig 9)

Infectious hepatitis

Among the 40 patients with infectious hepatitis examined (Fig 10) the first galactose test resulted in an abnormal T ½ value in 23 cases (58)

Only ten patients showed an abnormal T ½ value after more than three weeks from the beginning of jaundice The mean duration of the disease in these patients up to the time when they could be discharged according to the general principles of the department was 54 days compared with 26 days for the remaining thirty patients The summed duration of the periods of acute disease and convalescence for eight of the patients with protracted abnormality of the galac

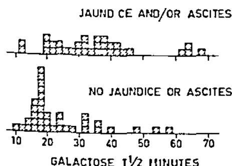


Fig 8 Distribution of galactose T ½ values in cirrhosis patients with jaundice and/or ascites and in patients without these signs Each square represents a test result

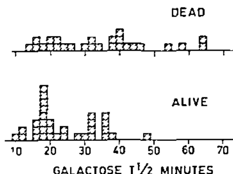


Fig 9 Distribution of galactose T ½ values in cirrhosis patients who died, and in surviving patients (see text) Each square represents a test result.

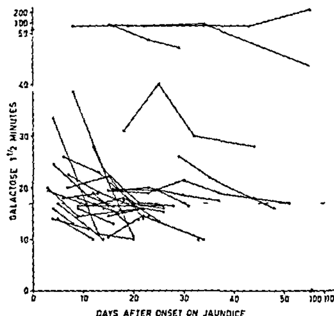


Fig 10 Gala tose T ½ values in patients with hepatitis in relation to the onset of jaundice Values from one and the same patient are connected by lines

tose test averaged 139 days and for the patients with rapid normalization of the galactose test 68 days

Two of the ten patients were lost sight of after their discharge from the hospital One patient who had T ½ values of 68 0 78 5 and 42 5 min died about three weeks after the last galactose test He had then been hospitalized for about four months

A follow up examination was made of the seven patients with protracted abnormality of the galactose test who were accessible and who had been under observation for more than six months It was found (Table I) that the galactose T ½ value was still abnormal in three patients 7–19 months after the acute illness and that cirrhosis of the liver was developing in at least one of them

Liver metastases

In this group of patients the galactose test showed abnormal T ½ values in eight cases (50 %) with

Table I *Follow up investigation of seven patients with hepatitis whose galactose test result was abnormal three weeks after onset of jaundice*

Sex and age	T½ value while in hospital (min)	Duration of acute disease + convalesc (days)	Follow up study: Months after acute disease	T½ value (min)	Bilirubin (mg/100 ml)	Thymol (U)	SGPT (U)	Liver biopsy	Subjective feeling
♀ 31	20 5	45+30	38	11 5	0 9	1 9	5	Normal	Healthy
♂ 32	⅃0 0	46+61	38	16 5	0 9	0 1	8	Small collections of lymphocytes between liver cell trabeculae	Healthy
♂ 63	31 0–28 0	47+26	19	41 0	0 9	1 3	13	Some polymorphism of liver cells	Healthy
♂ 26	19 5–17 0	53+2	11	13 5	0 7	1 4	5	Normal	Healthy
♀ 54	74 0–209	57+299	8	20 0	0 7	0 9	45	Not permitted by the patient	Tired but able to work
			14	17 0	0 4	0 8	5		
♀ 64	63 0–47 0	30+ ~250	7	67 0	8 4	11 7	250	Chronic hepatitis with developing cirrhosis	Chronically ill
♂ 46	19 5–17 5	38+30	21	16 5	0 8	1 0	9	Normal	Tired but able to work

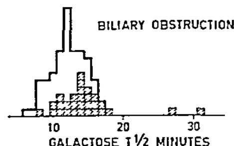


Fig. 11. Galactose T ½ values for patients with biliary obstruction. Notation as in Fig. 7.

a range of 17.5–38.0 min. There appeared to be no correlation between the T ½ value and the extent of the liver metastases.

Biliary obstruction

Of the 27 patients with biliary obstruction 25 showed normal values (93 %) and two abnormal values (7 %) at the galactose test. Twenty-six tests were made in the former group and three tests in the latter. The serum bilirubin value was above 10 mg/100 ml in five patients and all showed normal results at the galactose test. The distribution of all the 29 test results is shown in Fig. 11.

One patient with an abnormal T ½ value was a 79-year-old man who had been jaundiced for 30 days and had a large stone obstructing the common bile duct. A liver biopsy at operation showed, apart from biliary stasis, extensive focal liver cell necrosis and areas of fatty infiltration. His T ½ value was 27.0 min.

The other patient with abnormal T ½ values was a 63-year-old man with obstruction of the common bile duct due to a cancer of the pancreas, which was also interfering with the blood flow in the superior mesenteric vein. His first T ½ value obtained just over a week from the beginning of jaundice was 18.0 min. Two weeks later the T ½ value was 30.5 min. The liver biopsy in this case mainly showed biliary stasis.

Table II shows that all the galactose tests performed within a week after the onset of jaundice resulted in normal T ½ values with approximately the same distribution as in the normal case material. When more than a week had passed the mean T ½ value was higher though still normal both in patients with stone and cancer obstruction.

Table II. *Galactose T ½ values for patients with biliary obstruction of different duration*

Time from onset of jaundice	No. of tests	T ½ value Mean and range (min)
1–7 days		
Stone	11	1[illegible]3 (9.5–16.0)
Cancer	1	17.0
8–40 days		
Stone	9	16.0 (11.0–27.0)
Cancer	8	15.4 (7.5–30.5)
Total	29	14.5 (7.5–30.5)

The retention of galactose at 40 and 60 minutes

The values for the retained galactose concentration in the blood 40 and 60 min respectively after the injection were also tested as parameters of the galactose elimination. These concentration values both showed a reasonably even distribution in the normal case material.

The retention at 40 min was 20.5 ± 9.1 mg/100 ml (mean ± S.D.) and the corresponding value for the retention at 60 min was 7.3 ± 4.1 mg/100 ml. The coefficients of variation were ±44 % and ±56 % respectively, compared with ±22 %

Table III. *Normal and abnormal galactose test results given as the T ½ value and the galactose concentration at 40 and 60 min in patients with cirrhosis and biliary obstruction*

Diagnosis and test results	T ½ value Normal 12.0±2.6 min (n=75) No.	%	Gal. conc. after 40 min Normal 20.5±9.1 mg/100 ml (n=75) No.	%	Gal. conc. after 60 min Normal 7.3±4.1 mg/100 ml (n=7[illegible]) No.	%
Cirrhosis						
Normal	11	16	14	20	16	23
Abnormal	59	84	56	80	54	77
Biliary obstruction						
Normal	26	90	20	69	21	72
Abnormal	3	10	9	31	8	[illegible]8

for the T½ value The standard errors of single determinations calculated from duplicate tests were ±27% and ±36% of the mean retention value at 40 and 60 min respectively

When the mean value plus two standard deviations was used as the upper normal limit for the retention a greater number of patients with cirrhosis were classified as normal than when the T½ value was used as the parameter (Table III)

DISCUSSION

Conversion of galactose to glucose or glucose metabolites in the liver forms the main route for galactose elimination from the blood (for discussion see (8))

Renal excretion is the most important extrahepatic pathway When it is corrected for or when it is negligibly small the rate of galactose disappearance from the blood after intravenous administration must thus be a good indicator of the liver function

Infusion experiments (24 28) indicate that a atic maximal removal capacity for galactose about 500 mg/min in normal subjects is at a plasma concentration of about 50 mg/100 ml and that above that level the hepatic removal of galactose is thus independent of the concentration

Determination of the galactose removing capacity of the liver by such a method is too laborious for clinical routine Single injection tests are more suitable for this purpose

A very simple method for a single injection test is to determine the retention of galactose in the blood at a certain time after the injection (30) In the present investigation of this method a lower degree of reproducibility was found and greater overlapping between normal and pathological cases than when the whole elimination curve was considered

A semilogarithmic plot of the disappearance curve after a single injection of galactose was introduced by Colcher et al (4) who used the K value of the curve to express the elimination rate The T½ value for the galactose elimination which is another way of expressing the slope of the curve was used by Vink (25) The present method is rather similar to these two methods but contains some improvements the use of a specific method for the galactose analyses being the most important one In comparison with the previous reports a more extensive study of the method has been made here in some respects and the clinical use of the method in different liver diseases has been investigated in larger groups of patients with carefully verified diagnoses

In another single injection method Tygstrup (22) calculated the maximal hepatic removal of galactose from the slope of a linear part of the elimination curve This part was found after an initial mixing period and above blood concentrations of about 50 mg/100 ml The values obtained agreed rather well with those obtained in infusion experiments The comparison was essentially made in normal subjects however

It has been a matter of discussion whether the blood disappearance curve after a single injection of galactose is linear (22 24 27) or exponential (3 4 7, 20) i e whether the galactose removal rate is constant or dependent on the concentration

The infusion experiments (24 28) provide convincing evidence that in the liver the galactose metabolism is rate limited i e a linear function of the time above a certain concentration level But it is obvious that the elimination curve after a single injection of galactose is also influenced by other factors than liver metabolism e g distribution of the galactose between different compartments of the body (3) and urinary excretion These factors may contribute to make the elimination curve deviate from linearity In fact an unequivocal linear part of the curve was rarely obtained in this investigation (cf Fig 2)

Obviously the overall elimination of galactose from the blood after a single injection is not truly described by any of the simple functions considered It was empirically found however that in the present method the semilogarithmically plotted curve could easily be approximated to a straight line in most cases

The T½ value was not influenced by the age of the adult patients in this investigation In infants and children below the age of about 15 years galactose is more rapidly eliminated than in adults (26) The normal value determined here is therefore not valid at low ages

The dose of galactose for the test 350 mg/kg body weight, was chosen in order to make the galactosuria negligibly small and yet to obtain blood galactose values fitting the sensitivity of the analytical method It was also considered an advantage to keep the galactose concentration of the solution below a level that could cause damage to the venous endothelium and to give a volume of fluid that could be tolerated by most patients It was confirmed that the urinary excretion percentage was essentially identical in patients with cirrhosis and in normal subjects in spite of the retarded disappearance of the galactose from the blood in the former group (21)

Stenstam (20) claimed that the administration of glucose simultaneously with the galactose improved the metabolism of galactose in normal people but probably not in subjects with an abnormal liver In this investigation no significant influence upon the galactose elimination was observed when an equal amount of glucose was added to the galactose Therefore it does not seem probable that small glucose impurities in commercial galactose solutions would be of importance for the result of the galactose test.

Abnormal $T^1/_2$ values were not obtained in all the patients with a histologically verified cirrhosis of the liver This was not unexpected as it is known that some degree of cirrhosis may occur without any appreciable reduction of the liver function

There were clinical indications however that the result of the galactose test was correlated to the severity of the cirrhosis higher $T^1/_2$ values were generally found in the presence of jaundice or ascites than in the absence of these signs generally associated with advanced cirrhosis Further the abnormality of the values was on an average greater in the patients who died than in the surviving group

The liver cell damage in hepatitis is pronounced even before the icteric phase (19) and generally it is soon restituted The first investigation on the hepatitis patients in this material was performed at varying stages of the icteric phase Although the percentage of abnormal $T^1/_2$ values would probably have been higher if all the tests had been made on one of the first days of jaundice it is obvious that as a rule the galactose metabolizing function of the liver was only moderately impaired This is in accordance with the general concept of acute infectious hepatitis as a benign disease (5 9 17)

The galactose test may help to identify patients with a severe form of hepatitis in those whose $T^1/_2$ value was still abnormal after three weeks the duration of the disease was longer than usual and there was probably therefore a comparatively more serious liver involvement. This opinion is also supported by the fact that one of these patients died and another was seen to develop cirrhosis of the liver

The mainly normal results of the galactose tests in cases of biliary obstruction show that elevation of the serum bilirubin does not interfere with the metabolism of galactose The test therefore may be expected to give comparable estimations of liver function in icteric and anicteric patients The galactose test may also be used in the differential diagnosis between obstructive and parenchymatous jaundice as was suggested for instance by Bassett et al (2)

It is well known however that protracted biliary stasis causes liver cell damage In accordance with that fact the mean $T^1/_2$ value was higher when the duration of jaundice exceeded seven days than when it did not. Abnormal $T^1/_2$ values also occurred among patients with protracted jaundice

CONCLUSIONS

This galactose test is of value for the investigation of different liver disorders It may be recommended

(*a*) In patients with liver cirrhosis and other conditions of parenchymal damage of the liver in order to demonstrate significant reduction in liver function

(*b*) In patients at an early stage of jaundice to help to ascertain whether the cause is biliary obstruction or parenchymal damage

(*c*) In patients with prolonged obstructive jaundice as a liver function test in the preoperative evaluation of the surgical risk.

(*d*) In patients with acute infectious hepatitis during the second or third week of the disease for guidance as to the restoration of the liver

ACKNOWLEDGEMENTS

This investigation was supported by The Medical Faculty of Uppsala Uppsala and AB Kabi Stockholm

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STEROID DETERMINATIONS IN SIX CASES OF HYPERPLASIA AND THREE CASES OF TUMOUR OF THE ADRENAL CORTEX

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Abstract A report is given of nine cases of adrenocortical hyperfunction six of these were due to hyper plasia two to carcinoma and one to adenoma of the adrenal cortex The cases were subjected to tests of ACTH stimulation 11β hydroxylase inhibition with metopirone and suppression with dexamethasone In urine col lections obtained before the tests, the following corti costeroid metabolites were determined tetrahydro S preg nanediol pregnanetriol dehydroepiandrosterone aetiocholanolone androsterone aldosterone oestriol and free" cortisol Plasma cortisol values were obtained at 8 a.m and 8 p m

The experience gained in this series can be summarized as follows

Plasma cortisol determined at night is a useful screen in test in suspected adrenocortical hyperfunction The resting 17 ketosteroid (17 KS) and 17 ketogenic steroid (17 KGS) excretion is too inclusive for this purpose as slight elevations are seen in obesity and anxiety The occasional adrenal adenoma may furthermore be missed Suppression tests performed with dexamethasone and evaluated on the basis of corticosteroid determinations in plasma or urine conclude the screening procedures The patients who remain suspect of having adrenocortical disease subsequent to the screening procedures should be investigated with as complete a set of procedures as are available An extensive spectrum is necessary if a firm and differential diagnosis is to be made on the biochemical evidence

The ACTH stimulation has been of value in the differentiation between normal function and the excessive function of adrenal hyperplasia but not in the distinc tion between hyperplasia and tumours as the latter cases also responded with increases in output.

Metopirone is very useful in differential diagnosis, particularly if supplemented with tetrahydro S determina tion The tumour cases had elevated values of tetra hydro S in the control urine samples and showed no increase in 17 KS and 17 KGS during the test

Dexamethasone – mg q i d reduced the urinary 17 KS and 17 KGS in the hyperplasia cases but not in the tumours

The metabolites from the different "levels" of adrenocortical steroid synthesis were elevated at various degrees, and rather irregularly in the carcinoma cases In the cortisol producing adenoma the urinary metabolites of adrenal andro ens were excreted in subnormal amounts

During recent years an extensive spectrum of chemical steroid analyses has become available to clinicians at larger medical centres The applica tion of these analytical methods to the various forms of adrenocortical hyperfunction has been of help to the clinician in explaining some of the pathophysiology of the conditions and in provid ing diagnostic tests The proper application of these tests and their interpretation and evaluation are current problems

Lipsett and Wilson (20) gave a clear account of the abnormal quantitative distribution of urinary steroid metabolites in ten cases of adrenal cortical carcinoma They emphasized the value of the metabolite tetrahydro S as a specific indicator of carcinoma an observation made earlier by Jailer et al (16) Pal and James (28) demonstrated an enormous increase in DHA and pregnenetriol in the urine of one case of adrenocortical car cinoma a less marked increase in another case and moderate increases in several cases of hyper plasia In the case described by Harrison et al (15) the high DHA excretion was combined with an increased output of oestrogen Martin and Hamman (21) determined the metabolites preg nanedial and pregnanetriol before and after ACTH stimulation They found the results of great help in assessing the aetiology of Cushings syndrome thereby supporting the findings of Damkjær Nielsen et al (6) In view of the wide spectrum of steroids normally secreted from the adrenal cortex (8) and the number of enzymatic systems involved it is possible that similar ob-

servations can be made regarding other metabolites

The other approach to the differential diagnosis of the various causes of Cushings syndrome is provided by tests of the functional capacity of the pituitary adrenal axis with use of ACTH dexamethasone and metopirone Birke et al (2) demonstrated an enhanced response to ACTH in adrenal hyperplasia but reduced responses in adrenal adenoma and carcinoma the parameter of the measurement being the urinary excretion of 17 hydroxycorticosteroids Ernest (14) on the other hand did not find this test particularly helpful in the differentiation of diagnoses The suppression test using dexamethasone at the dose levels of 0 5 mg or 2 mg q i d was found very useful by Liddle (18) in a large series of patients Others (13 17) maintain that the response to the test must be evaluated with caution as misleading results can be obtained particularly in nodular hyperplasia Irregular responses may also occur due to marked variation in basal excretion of steroids Inadequate specificity of the methods generally used for the determination of urinary corticosteroids may also give rise to misleading results The evidence presented by Jailer et al (16) that the usefulness of the metopirone test for the diagnosis of adrenal carcinoma is dependent upon the simultaneous determination of tetrahydro S and 17 ketogenic steroids is supported by our observations (26) An excellent survey of the various tests of function has recently been presented by Cope (5) Ross et al (31) have successfully related corticosteroid analyses and tests (or at least some of them) to clinical and biochemical aspects of Cushings syndrome

In the cases to be presented in this paper a rather wide spectrum of urinary steroids has been determined as well as the response to ACTH metopirone and dexamethasone This then allows a certain comparison and evaluation of the merits of the different analytical approaches The diagnoses have been verified by operation

MATERIAL

A survey of the case material is presented in Table I Cases 1 and 2 had large carcinomas case 3 was found to have a small adenoma, the remainder has been classified as bilateral hyperplasia.

METHODS

ACTH stimulation

ACTH in the form of a long acting preparation (Jaton Prolongatum A L.) was given intramuscularly in a dose of 1 0 IU at 10 p.m. for a sequence of three days. The urine collections started at the time of injection and continued for the following 24 hours 17 KS and 17 KGS determinations were performed in the three 24 hour urine collections during stimulation as well as in samples from the two 24 hour periods preceding the ACTH administration The results presented in this article were obtained on the third day of stimulation.

Metopirone test

Metopirone was given at 8 a.m., 10 a.m 12 a.m 2 p.m 4 p.m 6 p.m., 8 p.m and 10 p.m. at a dose level of 250 mg this sequence of administration was continued with 750 mg at midnight and at 4 a m The total dose therefore amounted to 3.5 g within 24 hours Urine was collected for 24 hour periods before during and after the metopirone administration. In these urine collections 17 KS and 17 KGS as well as the metabolite tetrahydro S were determined The figures shown in the tables are from the first and the last day of the test

Dexamethasone suppression

Dexamethasone was given at two dose levels 0 5 mg q.i.d. for two days being followed by 2 mg q.i.d for two days Urine was collected for 17 KS and 17 KGS determination during administration as well as for the two days preceding the test The figures presented in the tables were obtained on the last day of each dose

Chemical methods for steroid determination (in outline)

17 KS and 17 KGS were determined by the method of Norymberski et al (27) as modified by Diczfalusy et al (7) The Zimmermann reaction was performed using the organic base N benzyl trimethyl ammonium methoxide according to Bongiovanni et al (3)

Tetrahydro S was measured by the Porter Silber reaction after initial hydrolysis with β glucuronidase chloroform extraction, purification of the extract, and chromatography in the systems toluene/propylene glycol followed by a modified Bush B_5. The procedure is described in more detail elsewhere (24)

Oestriol was measured by the modified method of Eberlein et al (10) based on the original procedure of Brown and Bauld

The procedure for aldosterone closely resembled the method of Neher and Wettstein (23) but the chromatography was continued in a third system, Bush B_4 and quantitation obtained by the isoniazide colour reaction (33) Further details of the procedure have been presented elsewhere (25)

In the cases of pregnanediol and pregnanetriol the urine samples were hydrolyzed with β glucuronidase and extracted with chloroform. After purification and evaporation of the extracts, chromatography was performed on paper with petroleum ether (100 –120)/toluene (1) as the mobile phase and 85 methanol in water as the stationary phase The final quantitation was achieved with the bisulfite sulfuric acid reaction (9) For pregnan-

Table I *Survey of clinical features and pathological anatomy of the adrenals removed at operation*

Case	Age	Sex	Symptoms and signs	Adrenal pathology	Adrenal weight
1	24	♀	Moderate hirsutism for 2 years Metrorrhagia No features of Cushing s syndrome Four years postop without recurrence	Encapsulated irregular tumour large areas of necrosis nodules 4–5 cm diameter with cells slightly resembling zona fasc marked criteria of malignancy in cell nuclei	1700 g Right side
2	6?	♀	For 6 months moderate hirsutism Periods of failing mental orientation Incipient features of Cushing s syndrome	Encapsulated tumour with necrosis studded with pea sized nodules of carcinomatous cells of rather low differentiation	480 g Right side
3	31	♀	Tendency to oedema formation during 3 years Oligo menorrhoea Development of featur s of Cushing s syndrome during period of observation	Encapsulated tumour cells resembling those of zona reticularis well differentiated Marked atrophy of cortical tissue adherent to tumour	9 5 g Left side
4	28	♀	For 1 5 to 2 years typical features of Cushing s syndrome complete Amenorrhoea for 6 months	Slight hyperplasia right side left side areas of necrosis Fragment of right adrenal left in situ	Right 5 2 g Left 5 0 g
5	33	♀	For 6 months typical features of Cushing s syndrome in complete form	Histological signs of hyperplastic overactivity both sides Fragment left in situ on right side	Right 7 4 g Left 6 3 g
6	49	♀	Diagnosed Cushing s syndrome for 15 years Right adrenal removed 14 years ago admission for recurrence	Histologically irregular hyperplasia both sides	Left 12 9 g
7	45	♀	Typical features of Cushing s syndrome for 6 years	Hyperplasia with signs of ACTH stimulation both sides Fragment of left gland left in situ	Right 7 9 g Left 6 3 g
8	61	♀	Hirsut sm and reddish-cyanotic colour of face increasing during last 2 years	Bilateral hyperplasia hyperplastic nodule	Right 6 0 g Left 6 3 g
9	45	♂	Features of Cushing s syndrome for 5 years	Bilateral hyperplasia signs of active ACTH stimulation	Right 1 0 g Left 14 2 g

ediol the colour was read at 390 4₂5 and 460 mμ for pregnanetriol at 405 440 and 475 mμ the specific ex tinctions bein₀ obtained by application of the correction of Allen (1)

To determine dehydroepiandrosterone aetiocholanolone androsterone the urinary sulphates were precipitated with barium chloride the urine centrifu₀ed and the super natant incubated with a mixture of β glucuronidase and sulphatase (Glusulase Endo Laboratories) for 48 hours extra enzyme being added after ?4 hours of incubation After an initial extraction with ether the urine was boiled with hydrochloric acid and re-extracted The combined ether extract was washed with ? N NaOH and distilled water evaporated to dryness and subjected to paper chromatography in the system isooctane/methanol/water (10 9 1) The ketosteroids were stained on the paper and the selected *coloured spots eluted according to the* method of Epstein and Zak (1) Quantitation was ob tained by photometry at 430 515 and 600 mμ with the application of the correction of Allen (1)

The urinary free cortisol was determined after a procedure of hydrolysis extraction and chromatography corresponding to that of aldosterone in these cases Plasma cortisol" was measured by a method similar to that of Peterson et al (30) For both of these cortisol determina tions the final measurement was made with the Porter Silber reaction reading at 370 410 and 450 mμ and using the correction of Allen (1)

The urine samples for the determination of the spec trum of steroid metabolites were collected shortly after admission to the hospital and before the patients were subjected to tests or treatment.

RESULTS

The amounts of the steroid metabolites excreted in the urine of the patients are presented in Table II Most of the figures are means of two values obtained in separate 24 hour urine samples a few are from one urine collection only and some are based on three or more collections The values for the same metabolite in each patient were usually in close agreement and always in the same range It is to be noted that the figures for pregnanediol and pregnanetriol cas were

Table II *Spectrum of steroid excretion in carcinoma adenoma and hyperplasia of the adrenal cortex determined in the resting state*

	Carcinoma		Adenoma	Hyperplasia Cases 4–9		Normal
	Case 1	Case 2	Case 3	Mean	Range	Range
17 ketogenic steroids mg/24 h	37 9	50 7	16 7	36 9 (6 cases)	65 6 23 1	5–15
17 ketosteroids mg/24 h	42 5	24 6	2 8	14 0 (6 cases)	28 1 7 0	5–15
Pregnanediol mg/24 h	46 9[a]	3 7	0 5	1 9 (3 cases)	2 2 0 6	<1[b]
Pregnanetriol mg/24 h	46 3[a]	1 4	0 2	0 3 (3 cases)	0 6 0 1	<0 5[b]
Tetrahydro S mg/24 h	5 9	7 5	0 58	0 26 (5 cases)	1 2 0	<0 2
Aldosterone μg/24 h	8 1	24	5–28	8 1 (3 cases)	14 0 4 6	5–12
Dehydroepiandrosterone mg/24 h	8 5	2 3	0 1	1 1 (5 cases)	5 1 0 3	0 2–1 5
Androsterone mg/24 h	22 0	1 9	0 1	2 0 (5 cases)	4 0 0 8	1 5–4 5
Aetiocholanolone mg/24 h	10 4	10 7	0 5	4 3 (5 cases)	8 9 1 4	1 5–4 5
Oestriol μg 24 h	81	256	11 0	17 1 (2 cases)	27 3 9 8	<10[b]
Urinary free cortisol, μg/ 4 h		80		6? 5 (2 cases)	80 0 44 5	<30
Plasma cortisol Morning	27	36	37 6	28 5 (4 cases)	44 0 21 6	15–28
Night		40	41 6	36 5 (4 cases)	64 0 22 2	5–10

[a] Determined in a different urine sample separated from the rest of the analyses in this case by a time interval of 6 weeks
[b] Range for males and women without cyclic ovarian function.

obtained in a different urine collection separated by six weeks from the urine collections on which the other results from this patient are based

The range of normal values to the right in Table II are modified slightly from the standard normal range in order to be more closely applicable to the presented group of cases The values for oestriol and pregnanediol are given without any consideration to the cyclic ovarian increases in output

As can be seen in Table II some of the parameters seem to be useful guides in separating the tumour cases as well as the hyperplasias from normal individuals The increased level of plasma cortisol at night with the resulting obliterated diurnal variation is common to both types of hyperfunction. The urinary free" cortisol also showed an increase in both types in the few cases in which it was carried out The 17 KGS excretion was above normal in all instances and in most of the cases very markedly so Extensive overlap into the normal range was found for the plasma cortisol values obtained in the morning and also for the 24 hour 17 KS excretion For the latter parameter this applies mainly to the hyperplasias

Distinguishing features in the two cases of carcinoma are the pronounced elevation of the tetrahydro S excretion and the high DHA aetiocholanolone androsterone and oestriol In case 1 the amount of pregnanediol excreted is higher than during the last three months of a normal pregnancy and pregnanetriol has a corresponding excessive rate of excretion These two parameters also show a clear increase above the normal in case 2 when age and lack of cyclic ovarian function are considered The adenoma case presents a different pattern tetrahydro S is only moderately increased and the excretion of 17 KS androsterone aetiocholanolone and DHA is below normal Plasma cortisol is clearly elevated with absent diurnal variation the 17KGS excretion is on the other hand slightly above the upper normal range

Table III *Tumours functional tests*

	ACTH		Metopirone		Dexamethasone	
	Resting	Response	Resting	Response	0 5 mg × 4	2 mg × 4
Carcinoma (case 1)						
17 ketogenic steroids, mg/24 h	37 9	36 8	33 6	43 2		78 9
17 ketosteroids, mg/24 h	42 5	122 8	82 6	86 4		29 6
THS mg/24 h			5 9	14 9		
Carcinoma (case 2)						
17 ketogenic steroids, mg/24 h	50 7	107 3	38 9	37 8	61 7	63 8
17 ketosteroids, mg/24 h	24 6	37 4	13 9	18 3	30 5	25 0
THS mg/24 h			*7.5*	*15 3*		
Pregnanetriol mg/24 h	1 4	6 3				
Pregnanediol mg/24 h	3 7	7 6				
Aldosterone µg/24 h	[illegible]4					38
"Free cortisol µg/24 h (urine)	80					80
Oestriol µg/24 h	258	39.2				
Adenoma (case 3)						
17 ketogenic steroids, mg/24 h	16 7	31 6	[illegible]0 8	14 0	19 4	19 3
17 ketosteroids, mg/ 4 h	2 8	6 4	4 2	1 8	2 2	6 3
THS mg/24 h			0 53	2 0		
Plasma cortisol, µg/100 ml	33 6	50 6				

Table IV *Hyperplasia functional tests*

	Starting value (mg/24 h)		Response value (mg/24 h)		Percent variation response value × 100 / starting value	
	Mean	Range	Mean	Range	Mean	Range
ACTH 5 cases						
17 ketogenic steroids	34 6	55 2	104 8	136 0	304	560
		22.6		45 7		151
17 ketosteroids	10 4	16 9	25 7	37 7	247	390
		6 9		15 3		192
Metopirone 3 cases						
17 ketogenic steroids	32 3	38 6	69 5	105 1	215	272
		28 9		46 2		160
17 ketosteroids	15 1	19 1	20 3	22.2	134	215
		10 3		18[illegible]		107
Tetrahydro S	0 39	1.2	[illegible]0 4	40 5		
		0		9 7		
Dexamethasone 0 5 mg q i d 4 cases						
17 ketogenic steroids	45 9	65 6	31 4	39 6	77	91
		33 2		29 7		60
17 keto steroids	18 4	28 1	16 7	[illegible]7 3	93	121
		10 2		11 9		71
Dexamethasone 2 mg q i.d. 4 cases						
17 ketogen c steroids	45 9	65 6	25 5	41 4	54	63
		33		14 0		42
17 ketosteroids	18 4	28 1	12.7	18 6	74	88
		10 2		8 5		58

The results of the functional tests as applied to the tumour cases are presented in Table III In both cases of carcinoma there is a response to ACTH In case 1 this response is qualitatively abnormal being limited to the 17 KS excretion in case 2 the response is closely similar to that seen in hyperplasias involving both 17 KS and 17 KGS to the same degree Metopirone produces very little change in the 17 KS and 17 KGS values THS excretion is characterized by a markedly abnormal starting value and an increase above the normal range There is no suppression with dexamethasone in these cases The adenoma responds rather weakly to ACTH the resulting values from this stimulation are in the lower normal range Metopirone gives a fall in 17 KS and 17 KGS excretion and the THS output after metopirone is below the normal range (5–12 mg/24 h) There is no suppression by dexamethasone

In case 2 pregnanediol and pregnanetriol have been determined during the ACTH stimulation the distinct increase in the levels of these lends support to the assumption that they are of adrenal origin in this instance

The term exaggerated normal responses" would tend to describe the majority of the results presented in Table IV showing the functional tests in the adrenocortical hyperplasias In four out of five cases the stimulation values for 17 KS and 17 KGS are beyond the normal range after ACTH as they are for all three cases following metopirone In this latter test response the THS excretion is at the upper normal limit for one case and well above for the other two Dexamethasone at the high dose level (2 mg q i d) provides a fair suppression of 17 KGS output (37–58 %) but it is to be noted that some suppression (9–40 %) is also obtained by the lower dose of 0 5 mg q i d As for the response in 17 KS excretion there is a mean suppression of 9 % on the low dosage and 26 % on the high dosage

DISCUSSION

The complete set of tests as presented in this paper is obviously too large to be useful as a tool in handling the primary problem of the diagnostic unit namely to establish the probability of adrenocortical disease A small set of screening tests that single out patients for further study and possibly treatment is clearly needed For this screening purpose the determination of 17 KS and 17 KGS in the 24 hour urine collection of an unstimulated patient has been widely used As can be seen from the presented results in Table II the method would have clearly placed the carcinoma cases as well as the hyperplasias into the group for further study This cannot be said quite so safely for the adenoma case her excretion values for 17 KGS being just above the normal limit This sort of value is too frequently found by this method It occurs in young obese individuals and may easily be brought on by very slight emotional stimuli as for example admission to a hospital It is not possible to act with an all out diagnostic effort on this evidence alone

The danger of depending on the plasma cortisol determination for screening when performed on a morning sample is evident from the figures in Table II Some of these cases have values in the normal range As pointed out as early as in 1956 by Lindsay et al (19) and Mellinger and Smith (22) the obliterated diurnal variation in steroid excretion (and plasma concentration) is a good sign of adrenocortical overactivity this rather than the elevated values is the important feature as shown in the cases of Ekman et al (11) As can be seen plasma cortisol in the evening sample was always elevated in our cases In our hands however this test gives a number of positive responses which are not later supported by the accumulated evidence even if this is less pronounced than for the 17 KS and 17 KGS determination Of these two tests the determination of the diurnal variation more efficiently excludes the young obese individuals from further study Brooks et al (4) also found the plasma cortisol morning values unreliable for screening They furthermore emphasized the need for at least two abnormal indices in a given case in order to distinguish from the situation in young people with generalized obesity and pink striae

We have recently been trying the short term dexamethasone suppression as described by Pavlatos et al (29) as a part of out spectrum of diagnostic tests This method is sufficiently simple to be suitable for screening purposes requiring at its minimum only one administration of dexamethasone (1 mg at 10 or 11 p m) and one plasma cortisol determination the following morning at 8 a.m at which time it should normally be

less than 5 μg/100 ml Our experience with this test has been encouraging although failure of suppression occurred in cases in which hyper andrenocorticism was not confirmed by the total evidence

In our opinion the patients that are still suspected of adrenocortical hyperfunction (irre spective of aetiology) following a preliminary screening by the 24 hour 17 KS and 17 KGS excretion by the determination of the diurnal plasma cortisol variation and by a short term dexamethasone suppression ought to be in vestigated with all available tests of the types outlined previously This will provide a fair in sight regarding the total pathophysiology of the adrenal cortexes of the patient and the associated pathology can be predicted reasonably well The responses to various diagnostic spot tests" are sufficiently irregular to be misleading as sole criteria in deciding upon the final diagnosis and treatment

In the separation between the carcinomas on the one hand and the hyperplasias on the other the urinary metabolites of intermediates from corticosteroid synthesis are of diagnostic signifi cance The quantity of these metabolites in the urine reflects a deficient synthesis of steroid hormones by the tumour tissue whether the decisive factor in this respect is enzymatic coen zymatic or circulatory in nature has not been finally established (20 32) The defective trans formation at various stages of the steroid synthesis in the large masses of tumour tissue does not seem to prevent adequate or more than adequate secretion of cortisol from the tumorous glands The increased amount of urinary free" cortisol in case 2 is *strong evidence for this One must* however proceed with caution when estimating cortisol excretion on the basis of the values for the plasma cortisol and the 17 KGS in the urine Plasma cortisol will include steroids such as compound *S* 6-hydroxycortisol and the tetra hydro metabolites of these and cortisol The 17 KGS in the urine will include such compounds as pregnanetriol tetrahydro S and others which may occur in large amounts in the urine from tumour patients

Further support for the assumption that cor tisol was secreted from the tumours is provided by the function tests The results of these are easily explained if one assumes that the pituitary ACTH producing cells have been suppressed by cortisol from the tumours This is perhaps best demonstrated in the metopirone test in which there is no increase in the steroid output The increase in THS excretion in the carcinoma cases following metopirone is not to be interpreted as reflecting a response to an increased output of endogenous ACTH It must rather be seen as the modified end product of the continuously se creting autonomous tumour tissue affected by the 11β hydroxylase inhibitor Both carcinoma cases responded to exogenous ACTH with an increase in the 17 KS and 17 KGS output

All the findings in the adenoma case are ex plained by assuming that this tumour secreted cortisol at a rate slightly exceeding the physio logical level probably even without abatement during the night In addition it is likely that the tumour was responsible for the aldosterone ex cretion This point is however debatable as the aldosterone excretion showed marked variations in response to alterations in electrolyte intake and to the administration of diuretics The non tumorous cortical tissue adherent to the removed tumour was thin and atrophic but contained small cortical cells filled with lipid In addition small clusters of cells were seen at the outer surface These were believed to be remnants of the zona glomerulosa

None of the tumour cases responded to exo genous ACTH in the period of time shortly following the operation The significance of this observation is obscured by the heavy cortico-steroid therapy during and after the operation Up to the present time case 2 has been in need of supportive corticosteroid therapy (i e for a *period of three years)*

The biochemical evidence for cortisol produc tion by the tumours contrasts with the clinical picture of the tumour cases As a group the signs and symptoms of Cushings syndrome were in distinct The adenoma case with the lowest steroid values developed some features of the syndrome during the six months of observation before operation The explanation may be that the cortisol from the tumour was balanced by a reduced secretion from the non tumorous adreno-cortical tissue during the early stages of the disease The period when cortisol was in excess in the organs of the tumour cases may therefore have been of short duration An explanation on

the basis of the assumption that the steroid secretion in the carcinoma cases consisted almost exclusively of products of low biological activity seems less satisfactory

ABBREVIATIONS AND TRIVIAL NAMES

Tetrahydro S THS = 3α 17α 21 trihydroxy 5β pregnane 20-one
Pregnanediol = 5β pregnane 3α 20α-diol
Pregnanetriol = 5β pregnane 3α 17α 20α triol
Pregnenetriol = pregn 5-ene 3β 17α 20α triol
Dehydroepiandrosterone DHA = 3β hydroxy androst 5 en 17-one
Etiocholanolone = 3α hydroxy 5β androstan 17-one
Androsterone = 3α hydroxy 5α androstan 17-one
Dexamethasone = 9α fluoro 16α methylprednisolone
Metopirone = Metapyrapone Ciba Su 4885 2 methyl 1 2 bis(3 pyridyl) 1 propanone
ACTH = adrenocorticotrophic hormone

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PERSISTENCE OF BLOOD CELLS IN URINE

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Abstract In clinical work the stability of blood cells in urine is a point of practical importance Provided bacterial contamination can be avoided, erythrocytes and leucocytes are well preserved in acidic urine of a specific gravity higher than 1.008–1.011 for at least 24 hours Whether sterile specimens are kept at room temperature in a refrigerator or at 37 C seems unimportant.

In alkaline urines, the degree of lysis increases with increasing alkalinity and the duration of time of exposure Leucocytes are especially susceptible to cytolysis in alkaline environments.

If bacterial contamination is present, unreliable results are obtained.

A comparative study of the number of erythrocytes and leucocytes excreted in overnight specimens and in specimens collected during 2 hours in the morning estimated per mm³ and expressed as excretion rates revealed differences which can probably be accounted for mainly by the random variability of haemocytometer counts

The stability of blood cells in urine is a point of practical importance in clinical work. The duration of the collecting period and the time elapsed between voiding and examination of the specimen are involved.

In the quantitative determination of blood cells in urine Addis (2) recommended that collection of specimens be started in the evening, the whole night portion being collected at one voiding 12 hours later Long collecting periods have several disadvantages (13) including the possibility of more degeneration of cells. Shorter collecting periods have been recommended by several authors (12, 13 15) the period suggested being usually limited to 3–4 hours with or without fluid restrictions

This paper deals with the lysis of blood cells in urines of various specific gravities pH values and temperatures in laboratory experiments and the possible influence of the duration of the collecting period on the clinical material.

PERSISTENCE OF ERYTHROCYTES SUSPENDED IN URINE

Influence of specific gravity

Two urine specimens of specific gravity 1.030 and two of specific gravity 1.020 were diluted with distilled water giving two dilution series with specific gravities falling stepwise by 0 003 within the range 1.030–1.003 and two falling stepwise by 0 002 within the range 1.020–1.002. In the examination of large volumes of the undiluted specimens less than one erythrocyte/mm³ was observed. By the addition of venous blood (4 75 mill. erythrocytes/mm³) a count of about 475 erythrocytes/mm³ in the suspensions was aimed at. The pH of the various specimens in the four dilution series ranged from 5.6 to 6.4

The specimens were kept on the bench at 20 C for three hours, thoroughly mixed and transferred to an improved Neubauer haemocytometer Counts were made in a volume of one mm³ (in the large central square and the four large corner squares of the rulings in both chambers)

Results

Incipient lysis probably occurred below a specific gravity of 1 012. Considerable lysis was observed below a specific gravity 1 009 becoming almost total at the lowest specific gravities (Fig 1)

Influence of pH

Samples with pH ranging from 5.5 to 8.2 were examined Clean voided specimens were obtained from persons without diseases of the urinary tract. Alkaline specimens were obtained by addition of 0 1 N NaOH The final specific gravities ranged from 1.018 to 1.025 where hypotonic haemolysis could be disregarded By the addition of blood a count of 475 erythrocytes mm³ was aimed at. Specimens were kept at 20 C counts were made after 3 6 10 and 24 hours. The samples were sterile

Results

In acid specimens no loss of cells had occurred after 24 hours. With increasing alkalinity increasing degrees of lysis were observed (Fig. 2)

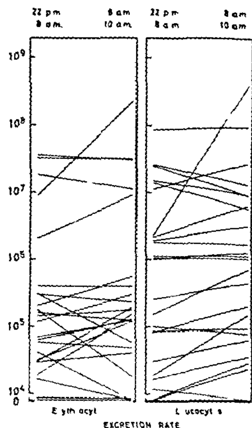


Fig 7 Excretion rate per hour of erythrocytes and leu ocytes in overnight specimens (10 h) compared with the consecutive morning specimen (h)

mm³ of urine in overnight specimens and 2 hour morning specimens the corresponding excretion rates being shown in Fig 7 In most cases the differences were small Only the alkaline urine showed a considerably higher number of cells in the 2 hour morning specimen (233 and 16,210 leucocytes/mm³ respectively in the two samples)

DISCUSSION

Although determining the degree of cell destruction by microscopic examination is an inaccurate procedure the method has been used in haemolysis studies (4 25 30) as well as in studies of lysis of erythrocytes in urine (31) The present study revealed pronounced lysis of erythrocytes in urines with specific gravities lower than 1 009 which presumably would correspond to osmolalities below a value of about 300 mOsm/kg Strauss (28) reported that erythrocytes in urine haemolyzed at freezing points higher than −0 36 C corresponding to an osmolality close to 200 mOsm/kg About half of the urinary osmolality is however accounted for by compounds which have no influence on the equilibrium between red cells and urine urea being of main importance (7 14) Erythrocytes suspended in normal urine like those in the urine of patients with bleeding from the lower urinary tract showed incipient lysis below a specific gravity of 1 012–1 010 amounting to more than 80% at a specific gravity of 1 0075–1 0059 (31) When suspended in pathological urines passed from patients with various kidney diseases incipient lysis was noted at specific gravities of 1 0118–1 0068 and 80% lysis at 1 0100–1 0044 (32) The discrepancy was attributed to a lower sodium intake in patients with kidney diseases

The shape of the haemolysis curve corresponded roughly to that usually observed in hypotonic haemolysis where 80–90% of the haemolysis has been reported to occur within a narrow range of concentrations corresponding to a change in NaCl concentration of 0 1% or about 35 mOsm (19)

Our study confirmed previous observations (18 32) that in acidic unrefrigerated urines no significant loss of erythrocytes occurs during the first 24 hours provided sterility is maintained Contaminated specimens gave unreliable results probably due to the haemolytic activities of various bacteria Winter and Knauth (32) reported that erythrocytes lost some haemoglobin at pH 4 5 but were easily recognized as double contoured rings even after 24 hours In alkaline urines of a pH higher than 7 5 some degree of lysis was noted which developed more rapidly at low erythrocyte concentrations than at higher ones (32) Urines of pH >6 0 will lose carbon dioxide if exposed to the air with a consequent rise in pH (5 9 17) On long standing in ordinary glassware the pH of a solution has been shown to increase (22)

In sterile specimens the storage temperature seemed unimportant Cells undergo significant lysis when kept in a plastic bag at body temperature (1) Bacterial contamination may play a part

Haemolysis has been reported to occur in cases of haematuria (16) and when blood is added to urine (21) Contact with glass may contribute to this effect

Chlorhexidine disinfectants used to clean female genitals before obtaining urine specimens may contaminate the urine and cause lysis of erythrocytes while droplets of lanolin in the disinfectant may be confused with erythrocytes (6)

Microscopic evaluation of leucocyte degeneration is difficult especially where cytolysis is partial and will introduce an important variable into quantitative investigations of urinary sediment In the present study a leucocyte was regarded as lost when it was felt that it would not with certainty have been recognized as such in an ordinary urine specimen This is admittedly an inaccurate method of differentiation

The lysis of leucocytes in various anisotonic media has been subject to several investigations (22 23 27 29) Generally leucocytes are found to be osmotically more resistant than erythrocytes In routine examinations of urinary sediment the required degree of urine concentration must be determined by the less resistant erythrocytes

In acidic and in neutral urines no loss of leucocytes was found during the first 24 hours At pH 7 3 a slight reduction of the number was noted after three hours With increasing alkalinity lysis developed more rapidly until at pH 8 4 the majority of the leucocytes disappeared in the course of a few minutes

As early as 1839 Rayer (20) observed that leucocytes disappear rapidly from alkaline urine and the futility of counting leucocytes under these circumstances has been emphasized repeatedly (3 11) At pH values above 6 8 a greater and more rapid destruction of leucocytes has been noted (26) Contamination by Proteus causes a rapid rise in pH with a concomitant fall in the leucocyte count (10)

Leucocytes in sterile and acidic urine were well preserved for 24 hours in a refrigerator (Fig 5) At 20 C a slight loss was observed after 10 hours At 37 C we observed some loss of cells during the first 6 hours Subsequent counts showed decreased numbers and after 24 hours lysis was almost complete The pH had by this time increased to 8 5 Houghton and Pears (13) reported however a decrease of almost half the initial leucocyte number after 4 hours at 37 C while no loss occurred during observation for 8 hours at 22 C No information regarding pH was given McIntyre and Mou (18) reported that it is rare to find increased amounts of erythrocytes and leucocytes in urines with pH values of 7 or more

A comparative study of the number of cells in overnight specimens and in specimens collected during 2 hours in the morning revealed minor variations (Fig 7) In one urine the number of leucocytes was much larger in the morning specimen than in the overnight specimen This urine was heavily infected with Proteus vulgaris and the pH was 8 8 The discrepancy may therefore be attributed to intravesical lysis The other specimens showed an acidic reaction In conformity with other observations (24) it was found that the volume excreted per hour was larger in the morning than during the night but the difference was small The main variable is probably related to the random variability of haemocytometer counts (8) and provided the specimens are acidic and sufficiently concentrated the influence of cellular degeneration in the bladder before voiding is unimportant

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LATENCY TIME MAXIMAL AMPLITUDE AND ELECTROMYOGRAPHY IN DIABETIC PATIENTS

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Abst act The results are given of a study of three motor components in a selected material of young and elderly diabetics and a control group 1 the latency time ankle extensor dig brevis muscle by stimulation of the peroneal nerve electromyographic findings in the extensor dig brevis muscle and 3 the maximal amplitude of the extensor dig brevis muscle and the anterior tibial muscle by stimulation of the peroneal nerve

Signs were found of a rapidly appearing motor neuropathy (most pronounced in the younger age group in which the beginning of the diabetes is well defined) together with a further degeneration with increasing duration of the diabetes It was impossible to demonstrate any connection with the metabolic control

The development pattern demonstrated corresponds to that shown in a previous study in which the motor conduction velocity was used as parameter for the motor function.

Some of the results obtained suggest that the nerves of the younger diabetics have a greater ability to regenerate than those of the older patients

In a previous study the author demonstrated that the conduction velocity of the motor nerves gradually becomes reduced with increasing duration of the diabetes Also that this feature is most pronounced in young diabetics and that the control of the diabetes appears to play a part (5) The present investigation is concerned with the function of a peripheral muscle and the terminal parts of the nerve Only a few systematic studies are available in the literature on this subject and these investigations are based upon materials that only include a few younger patients

MATERIAL AND METHODS

The persons studied were the same as those described in a previous publication on the motor conduction velocity i e 46 non-diabetics and 144 diabetics (5) An addition has been made of a further 1 patients with diabetes mellitus The material thus consists of 46 non-diabetics (?4 men and 2_ women) without signs of peripheral nerve disease or other somatic disease of any importance together with 165 diabetics (99 men and 66 women) selected at random 100 from the medical outpatient clinic 50 from the medical department, 12 from the neurological department and three from the ophthalmological department

The material has been divided into two age groups 15–44 years and 45–74 years The diabetics have in addition been divided into three groups according to the duration of their diabetes Control of the metabolic status was evaluated according to the method described in the earlier paper (5)

The latency time from the stimulation of the peroneal nerve at the ankle and until the occurrence of a muscle potential in the extensor digitorum brevis muscle was measured by the method previously described (5) The muscle potential was registered via three coaxial needles placed in the muscle No importance was attached to the initial deflection of the muscle potential being positive or negative as it could be seen that this had no influence on the latency time thus measured

Electromyography was performed with the same three coaxial needle electrodes (DISA 13 No3 outer diameter 0 65 mm, leading-off surface 0 07 mm²) The muscle potentials were amplified and recorded on film by a DISA-electromyograph (input impedance 100 megohms shunted with 60 pF frequency band _–10 000 cycles/sec) The needles were placed in the extensor dig brevis muscle at intervals of 5–10 mm and moved out fanwise so that a total of 27 sites in the muscle were studied Attention was paid particularly to the action potential pattern at maximum voluntary effort (interference mixed pattern, single oscillations, or no activity) and the occurrence of denervation potentials but the average potential duration and the appearance of polyphased potentials were also measured according to Buchthal (1) It should however be noted that no attempt was made to obtain any definite number of measurable potentials or to reproduce them several times Only the following two types of photographically registered potentials were accepted as denervation potentials 1 Diphasic fibrillation potentials with two or three phases positive onset, duration 1–5 msec and amplitude of more than 50 μV 2 positive positive

Table I *The average latency time ankle extensor digitorum brevis muscle in non-diabetics and diabetics in milliseconds*

	Duration of diabetes			(y)
	Non-diab	<5	5–14	>15
Age 15–44				
Latency time	4 89 ±0 63	5 09 ±0 91	5 [illegible]6 ±0 99	5 65 ±0 94
No of pats	29	36	18	30
Average conduction distance (cm)	6 [illegible]	6 5	6 5	6 4
Age 45–74				
Latency time	4 48 ±0 65	5 16 ±0 89	5 24 ±1 03	4 87 ±0 66
No of pats	17	21	20	19
Average conduction distance (cm)	6 3	6 2	6 1	6 2

sharp waves with a duration of 4–8 m sec and an amplitude of more than 30 μV (2)

An abnormal electromyogram was defined as the occurrence of such denervation potentials at more than one of the 27 sites investigated in the muscle or an action potential pattern less than interference

Maximal amplitude An attempt was made to estimate the number of functioning muscle fibres in the extensor dig brevis muscle by stimulating the peroneal nerve at the capitulum of the fibula and measuring the peak to peak amplitude of a potential between two non insulated needle electrodes of 2 cm length and 0 47 mm diameter one being placed subcutaneously over the belly of the muscle and the other subcutaneously over the tendon further distally The stimulus was always supra maximal three times the threshold value The maximal amplitude of the anterior tibial muscle could be registered simultaneously using the same technique

All measurements were carried out at room temperature The left leg was used for the study with the exception of seven patients in whom this was impossible owing to ulcers amputation or other causes.

RESULTS

Latency time

The average latency time from the time of stimulation at the ankle and until the beginning of the muscle potential in the extensor digitorum brevis muscle is given in Table I for the different patient groups

The average conduction distance was almost equal in comparable groups and was not considered to explain the differences demonstrated

No difference was found between men and women

It is seen that the average latency time in the group of young patients is shorter in non-diabetics than in diabetics It increases with increasing duration of diabetes The increase is not large enough to produce a statistically significant difference between the various juxtaposed groups but there was however a significant difference between the young diabetics with a diabetes duration of less than five years and those of more than 15 years ($p < 0\,02$) Similarly a significant difference was found between the non-diabetics and the diabetics taken as a whole both for the

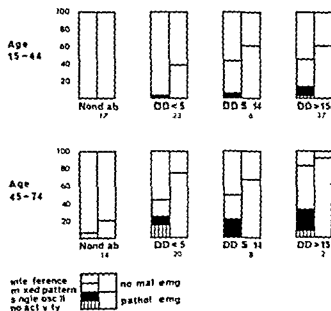


Fig 1 Incidence of loss of motor units and abnormal EMG of the extensor digitorum brevis muscle according to definition in text, in non diabetic persons and diabetics of different age and duration of diabetes

Table II *The results of EMG of the extensor dig brevis muscle*

	Age 15–44				Age 45–74			
		Years duration of diabetes				Years duration of diabetes		
	Non-diab	<5	5–14	>15	Non-diab	<5	5–14	>15
No of pats	17	23	16	37	14	20	18	12
Interference	17	22	9	20	13	11	9	2
Mixed pattern	0	0	6	12	1	4	5	6
Single oscillations	0	1	1	4	0	2	4	3
No activity	0	0	0	1	0	3	0	1
No of pats with ≥2 denerv pot	0	8	6	12	2	8	5	4
Average duration of pot in m sec	10 4	11 0	10 9	12 2	10 1	11 0	10 5	10 8
Average polyphasic potentials	10	11	9	17	7	13	11	7

young and for the older age groups ($p<0\ 002$ and $p<0\ 005$)

Electromyography

Electromyography was performed on 31 non diabetics and 126 diabetics The results are shown in Fig 1 and Table II It can be seen from Fig 1 that signs of denervation of the muscle are noticed very shortly after the clinical appearance of the diabetes mellitus and that this increases with the duration of the diabetes In the young age group 40 showed an abnormal EMG even before having had diabetes for five years The greatest number and most severe loss in motor units was found in the older age group

It can be seen from Table II that an increase in the average potential duration or increase in the number of polyphased potentials is noticeable only in the younger age group with diabetes of long duration Electromyograms which could be considered as suggesting myogenic affection have not occurred in the material nor have spontaneous polyphased giant potentials been observed

The number of subjects with denervation potentials was considerably higher among the diabetics than the non-diabetics but the duration of the diabetes in itself appears to have little influence on this

It was not possible to demonstrate a higher incidence of pathological EMG according to the definition employed in the younger diabetics with poorly controlled metabolic status than in the well-controlled patients in the same group with diabetes of short duration

Amongst the younger diabetics with diabetes of shorter duration than one year a pathological EMG was found in three cases out of 11 one case with single oscillations and two cases with too frequent occurrence of denervation potentials

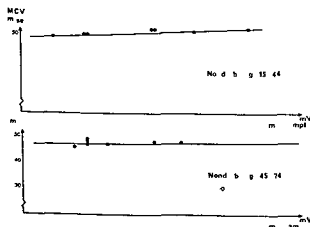


Fig 2 Relation between motor conduction velocity in the peroneal nerve and the maximal amplitude in the extensor digitorum brevis muscle in non diabetic persons

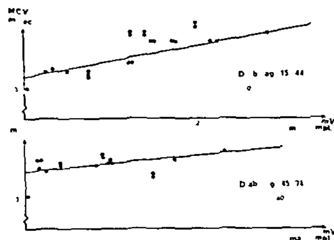


Fig 3 Relation between motor conduction velocity in the peroneal nerve and the maximal amplitude in the extensor digitorum brevis muscle in diabetics.

Maximal amplitude

The results of the experiments during which the amplitude of the potential was registered from the extensor dig brevis muscle by maximal stimulation of the peroneal nerve showed that even using subcutaneous electrodes this only gives a rough estimate of the function of the muscle with a very large dispersion of the results even in the normal material

In the two age groups of non-diabetics the values could not be shown to be correlated to the motor conduction velocity The regression coefficients are not significantly different from 0 (Fig 2) There was no age or sex difference

Fig 3 shows the corresponding results in diabetics A considerable number of values obtained in the patients are abnormal it is also seen that MCV and amplitude are positively correlated (regression coefficients significantly different from 0 $p < 0.001$ and $p < 0.02$)

Fig 4 shows that the potential amplitude in the younger age groups is significantly reduced after a diabetes duration of five years A correlation to the duration of the diabetes cannot be demonstrated for the older age group If the diabetics are considered as a whole a lower average maximal amplitude can however be seen and thus fewer functioning muscle fibres evaluated by this method than in the non-diabetics in this age group

Similar results were obtained by studying the anterior tibial muscle

DISCUSSION

As the majority of diabetic patients are elderly persons and as the most severe clinical pareses are most frequent in this age group it is natural that the majority of materials published contain only a few younger subjects



Fig 4 Average maximal amplitude of the extensor digitorum brevis and the anterior tibial muscles in non-diabetic persons and diabetics of different age and duration of the disease

In the present material the motor function of a peripheral muscle and the terminal nerve fibres has been studied in a representative material both of young and elderly diabetics with diabetes of various durations Even though the methods of measurement used are somewhat crude and the individual parameters do not give quite the same pattern there is a common trait the nerve muscle function in diabetics is poorer than that in the normal subjects and diminishes with increasing duration of the disease

The results obtained in the present *latency studies* are partly in agreement with those of Mayer (7) This author found an increased latency time in diabetics but only in the youngest age groups from 10–35 years

Our results are inconsistent with those of Mulder et al (8) These investigators found the same latency time from the ankle to the extensor dig brevis muscle in diabetics and normal subjects It should be noted however that there were only a few younger diabetics in the material

None of the above mentioned investigators have taken the problem of the duration of the diabetes into consideration In the material presented here it is demonstrated that the duration of the disease is of considerable importance for the measured nerve muscle function just as it is for other nerve functions (motor conduction velocity and vibratory sense) and for the different expressions of diabetic angiopathy (6)

Electromyographic studies in diabetes mellitus have been performed by several investigators Mulder and coworkers found signs of motor neuropathy in 38 of 80 patients but this figure includes patients with pathological maximal amplitude and latency time Exact figures for the occurrence of denervation potentials or loss of motor units are not given

Fagerberg and coworkers (4) studied several muscles by electromyography in 128 diabetics and found that the extensor dig brevis muscle was always affected when pathological findings could be demonstrated They found an increasing frequency of EMG abnormalities with increasing age and duration of the disease The later did not however apply to patients above 60 years of age In contrast to the material presented here denervation potentials were only found in seven of the 128 patients but the number of sites studied per muscle is not stated

Skillman and associates (9) carried out electromyography on 78 of the 103 patients in whom motor conduction velocity had been measured The EMG was pathological in 26 of the 78 patients In six cases there was an increased number of polyphased potentials and in 19 cases a reduced motor activity Denervation potentials were only found with severe pareses The age and duration of the diabetes is not stated for this material but the average age for the whole material of 103 patients was 52 years

Wiesendanger and Bischoff (10) studied 263 muscles in 54 diabetics with clinical signs of neuropathy These authors were able to demonstrate a certain difference in the EMG from the proximal and the distal muscles such that polyphasia and short potential duration were most frequent in the proximal muscles while fibrillation potentials were most frequent distally A pure myogenic picture was found in two patients

The present study confirms the findings of Fagerberg and coworkers on the relation between the duration of diabetes and EMG abnormality particularly in the young age group but in addition shows that it is often possible to find signs of denervation before five years duration of the disease This is in agreement with the previously demonstrated reduction in the nerve conduction velocity in patients with a short duration of the disease (5) The fact that the potential duration is not increased in this group of patients may be considered to indicate that the nerve affection is mainly peripheral in the beginning The late occurrence of an increase in the number of polyphased potentials may suggest that many years of diabetes are required before considerable sprouting takes place from the diseased nerves This hypothesis is also supported by the histological studies of Coers and Hildebrand (3) These investigators found that the first morphological sign of a diabetic neuropathy was a change in the motor end plates only later was an increase in the collateral sprouting of the motor nerve fibres observed

The *maximal muscular amplitude* in diabetics has been studied by Mulder and coworkers who used surface electrodes and found an average value over the extensor digitorum brevis muscle of respectively 7 6 mV in normal subjects and 4 0 mV in diabetics

In the present study a considerable dispersion

of the results was found but the same pattern appeared as in the electromyographic studies (Figs 1 and 4) The most pronounced functional reduction is seen immediately after the diagnosis of the diabetes mellitus in the older age group while it apparently takes place at a later period in the younger patients The fact that the number of functioning muscle fibres seems to be retained longer by the younger patients is interesting when it is remembered that the motor conduction velocity is lower in the younger long term diabetics than in the elderly (5) This could be the result of more sprouting in the young Such an explanation is also in agreement with the demonstrated increase in the potential duration and polyphasia in the young diabetics of long duration

The study presented here of the motor function in diabetics intensifies the impression obtained from the previous study of the motor conduction velocity all the results obtained suggest that the diabetic neuropathy is a nerve disease which begins soon after the commencement of the diabetes and gradually progresses with the duration of the disease On the other hand it has not been possible by the methods used here to demonstrate any connection with the control of the metabolic status A better understanding of the slightly different and more severe affection in the elderly diabetics is hardly possible without thorough histological studies covering both age groups

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VIBRATORY PERCEPTION THRESHOLD AND MOTOR CONDUCTION VELOCITY IN DIABETICS AND NON DIABETICS

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Abstract The vibratory sense measured by biothesiometry was found to be correlated to age and sex in non diabetics.

In diabetics the vibratory sense was correlated to the duration of the diabetes both on the upper and lower extremities No difference was found between the sexes.

It was possible to demonstrate a reduction of the vibratory sense already within the first year of diabetes in younger patients with a well-defined onset of the disease The same fact has been demonstrated previously with regard to the motor conduction velocity

In young diabetics motor and sensory nerve functions diminish in parallel

Impairment of the tendon reflexes and disturbances of sensibility are recognized as the most frequent and most easily demonstrated signs of diabetic neuropathy Slight reduction in motor function cannot be demonstrated at the bedside but has often been demonstrated by the use of more time-consuming neurophysiological techniques (4 10 14 16)

Sensory and motor nerve function are both reduced *with increasing duration of the diabetes* (3 6 7 9 18) whereas there is still some uncertainty regarding the part played by the severity of the metabolic abnormality

It has been demonstrated in previous studies on the motor function of diabetic patients that signs of nerve involvement can be shown very early after the clinical appearance of the disease and that a certain correlation can be found between the motor nerve abnormality and the metabolic status (6 7)

The object of the present study has been to investigate further the correlation between the vibratory perception threshold (VPT) and the duration of diabetes to study more thoroughly the vibratory perception threshold after a very short duration of diabetes and finally to study the connection between vibratory perception and the motor conduction velocity (MCV)

MATERIAL AND METHODS

The material consisted of 70 non-diabetic individuals and 210 diabetic patients Among the non-diabetics there were 57 patients without signs of somatic disease of any importance and 13 normal subjects. A fasting blood sugar of less than 110 mg% (Hagedorn-Norman Jensen) and no glucose in the 24-hour urine could be demonstrated in all of these subjects Glucose tolerance tests were not performed The 210 diabetics were selected at random 187 from the Second Clinic of Internal Medicine and 23 from other departments of the Kommunehospital or from the medical department of the Aarhus County Hospital The material was divided into two age groups and the diabetics into an additional three groups according to the duration of their diabetes The number of patients in the various groups and their sex is shown in Table I

Amongst the 74 young diabetics with a short duration of the disease 49 were at the time of examination under treatment with insulin and these could be divided into two groups based upon the degree of control of the metabolic state according to previously mentioned principles (6) Of these 49 14 were estimated as well-controlled 35 as poorly controlled. At the time of the examination 37 young diabetics had had the disease for *less than one year* and of these 24 were still *untreated*

The motor conduction velocity was measured in the peroneal nerve as previously described (6)

The threshold for the vibratory perception was measured with a biothesiometer (Bio-Medical Instrument Company Chagrin Falls Ohio) (12, 17) the amplitude of the vibrations being indicated by a voltmeter The highest readable value is 50 volts. The measurements were carried out at room temperature with the subject in a quiet room The vibrating stylus was placed upon the pulpa of the left index finger or left great toe which supported the weight of the vibrator The amplitude of the vibrations was gradually increased until vibrations were felt, after which the voltmeter was read. The VPT is stated as the mean

various vascular anomalies in long term diabetes (11)

The significance of the duration of diabetes for the VPT demonstrated by Steiness (18) and Jersild and Lauritzen (9) has also been confirmed in the present study In addition it has been shown that this correlation applies not only to the great toe but also to the index finger These facts suggest that a slowly developing ischemic process is of importance for the development of diabetic neuropathy

It was however also found that a reduction of VPT is demonstrable *already within the first year of diabetes* in young diabetics a finding parallel to the one with measurements of the MCV in the peroneal nerve (6)

This early neurological abnormality must be considered to be of a metabolic nature and possibly comparable to the biochemical (5 20) functional (2) and morphological (1) abnormalities demonstrated in nerve tissue in experimental diabetes It seems unlikely that in the first year of diabetes abnormalities would be present in the nutritive blood vessels of the peripheral nerves which could produce the results observed in the present study However the whole question of early abnormality of the blood vessels in various organs is still sub judice (15 21)

In an earlier study (6) it has been demonstrated that the average motor conduction velocity is reduced in a group of poorly regulated diabetics in comparison to a group of well regulated patients with diabetes of the same duration In a study in progress we have observed that some degree of normalization of the MCV can be demonstrated in many cases if the individual patient is followed closely during the initial phase of insulin treatment (8)

In the present study in which the VPT has been used as parameter it has not been possible to show any difference between "poorly regulated" and well regulated" groups of patients However the fact that vibratory perception is also under the influence of the actual metabolic state has been demonstrated by Steiness measurements of VPT during ischemia (19) In this study the VPT variations during ischemia were found to be abnormal with elevated blood sugar becoming normal within the course of a few days by very precise normalization of the blood sugar and abnormal once again as soon as the 24-hour blood sugar level was allowed to increase even slightly

Several studies in recent years have shown that an abnormality of the motor nerve system is a common finding in diabetics In the present work it is demonstrated that the development of diabetic neuropathy in younger diabetics occurs in such a manner that the motor and sensory abnormality follow each other

Hitherto the sensory disturbances in diabetic neuropathy have attracted much more attention than the motor dysfunction This may perhaps be due to the fact that mild sensory abnormalities are easily demonstrated by simple clinical methods of examination while mild motor abnormalities require more complicated apparatus It is probably also of importance that the actual clinical *symptoms* occurring late in the development of the neuropathy are dominated by sensory symptoms The patients are inconvenienced much more by paresthesia loss of sensory sense and by pain than by a mild reduction of muscle strength which at any rate is unimportant for patients who are incapacited by other long term diabetic lesions such as retinopathy and nephropathy

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SELF INDUCED PROTEIN CALORIE MALNUTRITION IN A HEALTHY ADULT MALE

A Study of Plasma Proteins Free Amino Acids and Lipids

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Abst act Clinical examination and analyses of a number of blood constituents including proteins individual free amino acids and lipids were performed in a 34-year-old athletic male who first had been on a calorie-deficient diet for seven months and then on a diet which was extremely low in proteins and lipids for 143 days

The concentrations of the different blood constituents were essentially normal despite a marked weight reduc t on and other signs of advanced protein depletion His physical capacity was obviously reduced but he recovered very soon after the institution of an ordinary ve etarian d et

The obs rvations ind cate that a calorie and protein deficient diet which is poor in sodium can be tolerated remarkably well by a healthy individual with extensive protein depots in the form of muscle tissue

In recent years most of the biochemical research in the field of protein-calorie malnutrition has been devoted to studies in children This subject has been reviewed extensively by Waterlow et al (16) Biochemical changes in plasma tend to ap pear late and are variable Among the changes observed are low plasma levels of most of the essential amino acids (2 6) and of triglycerides pho pholipids and cholesterol (10 11) In adults total starvation for shorter periods induces an elevation of the levels of branched chain amino acids (13) A diet low in protein but rich in calories when given for 63 days however produced a gradual decrease in all the essential plasma amino acids except lysine (14)

The present report describes observations in a young athletic non-obese man who for one year was on a calorie-deficient diet and who for the last 143 days restricted himself to a diet which was also extremely poor in protein and fat Clinical observations and analyses of plasma proteins amino acids lipids and of nitrogen com pounds in the urine are reported

CASE REPORT

The 34-year-old subject studied is a student at the Uni versity of Goteborg and works periodically as a docker for economical reasons Since about the age of 18 he has been an active athlete with good results in general athletic sports During the last –3 years he has added muscle buildin to his training pro ram For six years he has been a lacto-ve etarian On January 1 1965 he omitted milk products from his diet and ceased his muscle buildin training During the following seven months his body weight decreased from 93 kg (hei ht 184 cm) to 78 kg This weight decrease seems to have been caused both by the change of diet and by the reduction in physical trainin

On August 1 1965 he decided to rinse his body by a further extreme change of diet Durin the followin 143 days he consumed only fluids consisting of juices of carrots pears, and apples in about equal amounts In addition to this he consumed small amounts of red beats cabbage and orange juice Between August 1 and August 15 he consumed about 1 5 l per day correspond n to about 800 calories During the followin 16 days he starved alto ether and drank only fresh well water On September 1 he returned to the juice diet but increased his consumption to about 3 l per day correspond n to about 1600 calories After ha ing read about his d etary experiments in a newspaper we contacted h m and were allowed to investigate him on November 15 and on December 21 Desp te repeated advice to stop this dietary experiment he kept to the juice diet until December at wh ch time he returned to a general ve etarian d et His wei ht was 60 kg on December 2?

The intake of calories carbohydrates p otein fat vitamins irons and other m ne als was calculated from data obtained from the Nutr tional Di ision Nat onal Institute of Publ c Health Stockholm Sweden One liter of the juice mixture was found to contain 550 calories 10 g of prote n and 3 g of fat, i e about 88% of the

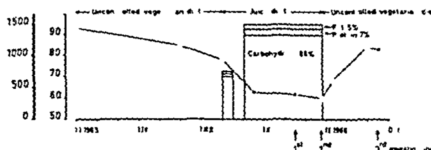


Fig 1 Body weight and calculated caloric intake

calories were supplied by carbohydrates In addition to this 1 l of the juice mixture contained about 5 mg iron 160 mg sodium 400 mg potassium 280 mg calcium 140 mg magnesium ? mg manganese 1 mg copper 280 mg phosphorus 50 mg sulphur and 60 mg chloride The vitamin content per l was 5000 IU of vitamin A 0 7 mg of B_1 0 4 mg of B_2 3 3 mg of niacine and 200 mg of vitamin C

The variations in weight and the calculated supply of calories carbohydrates proteins and fat are illustrated in Fig 1

During the juice diet period the subject's physical capacity decreased He used to run for at least one hour every day but at the end of the juice diet period he was not able to fulfil this training program for more than about ten minutes per day He was able to continue his studies however and felt that his intellectual capacity was unchanged He reported that his libido had disappeared after the two weeks of total starvation

At the general clinical examination no signs of any disease were observed The systolic arterial pressure was 100 mm Hg, the diastolic 60 mm Hg, and the pulse rate at rest about 60 min ECGs and X ray examination of the chest were normal There were no signs of edema

On December _ he decided to return to a general vegetarian diet including fruits, vegetables, beans, bread potatoes and vegetable margarine His body weight increased rapidly (Fig 1) and he was able to intensify his physical training very soon As early as January 7 1966 he started to work as a docker again and was able to perform heavy labour for 11 hours per day It should be noted however that he had temporary edema of his legs for 2-3 days during the first week of the general vegetarian diet This was the only time he had visible signs of edema He was investigated by us again on March 17 and was then obviously in a very good physical condition

CHEMICAL METHODS

Plasma concentrations of triglyceride cholesterol total phospholipids and free fatty acids were determined according to previously described methods (1?) Serum proteins were determined by paper electrophoresis plasma free amino acids by ion exchange chromatography (11) and urinary nitrogen constituents according to previously described methods (7)

RESULTS

The various blood constituents showed only small deviations from the normal (Table I) The hemoglobin concentration and the hematocrit values indicated a slight anemia during the juice diet period and the total iron binding capacity was also low Serum urea was decreased

The serum albumin level was somewhat low on the first two occasions and within normal limits at the time of the third investigation (Fig 2) The individual free plasma amino acid levels were essentially normal The alanine and glycine levels however were high and the proline level was somewhat low compared with the levels in nine healthy men of similar age (Fig 3) The alanine level was normal at the third investigation At that time the taurine level was low The total cholesterol and total phospholipid levels were low during the juice diet period whereas the triglyceride and free fatty acid levels were normal

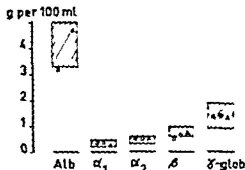


Fig Serum proteins at the first (□) second (O) and third (Δ) investigation. The shaded areas illustrate the normal range

Table I *Concentration of different blood constituents*

		Nov 15 1965	Dec 21 1965	March 17 1966	Normal range
Sodium	mEq/l	146	145	145	(138–148)
Potassium	mEq/l	3 5	4 2	3 9	(3 6–5 1)
Calcium	mEq/l	4 2	4 3	4 5	(4 3–5 3)
Chloride	mE/l	107	108	102	(99–107)
Total bicarbonate	mEq/l	27	29	29	(23–31)
Phosphate phosphorus	mEq/l	1 6	1 8	1 3	(1 6–2 6)
Hemoglobin	g/100 ml	13 8	11 7	14 5	(13–16)
Erythrocytes	mil /mm³	4 2	4 2	4 8	(4 5–5 5)
Hematocrit	vol.%	39	40	44	(41–50)
Serum iron	µg/100 ml	75	90	1 0	(80–180)
Total iron binding capacity	µg/100 ml	170	195	435	(250–400)
Total protein	g/100 ml	6 0	5 9	6 8	(6 0–8 0)
Creatinine	mg/100 ml	1 0	1 1	1 4	(0 8–1 4)
Urea N	mg/100 ml	6	10	10	(10–25)
Blood glucose	mg/100 ml	70	88	94	(70–110)

(Fig 4) During the restitution period there was a slight rise in the cholesterol level

The urinary excretion of nitrogen and creatinine was very low and no creatine was detectable (Table II) The excretion of amino nitrogen was in the lower normal range The urinary amino acid pattern was completely normal and there was no increase in the excretion of taurine and beta aminoisobutyric acid

Table II *Urinary excretion of total nitrogen urea nitrogen amino nitrogen and creatinine (mg/24 h)*

	Nov 15 1965	Dec 21 1965
Total nitrogen	3200	4780
Urea nitrogen	2050	3290
Amino nitrogen	60	53
Creatinine	940	830

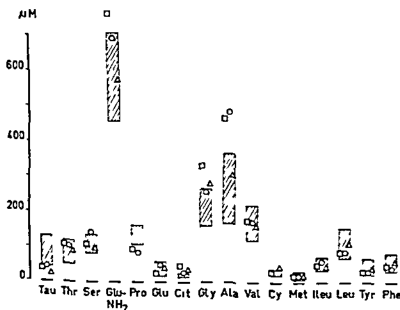


Fig 3 Plasma concentrations of taurine threonine serine glutamine proline glutamic acid citrulline glycine alanine cystine valine methionine isoleucine leucine tyrosine and phenylalanine at the first (□) second (O) and third (△) investigation The shaded areas illustrate the observed normal range in 9 healthy male individuals of similar age investigated with the same method in our laboratory

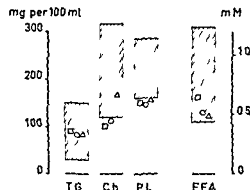


Fig 4 Plasma concentrations of triglycerides (*TG*) total cholesterol (*Ch*) total phospholipids (*PL*) and free fatty acids (*FFA*) at the first (□) second (○) and third (△) investigation. The shaded areas illustrate earlier observed normal range in healthy male individuals of similar age (1?)

DISCUSSION

The subject s keen interest in nutritional problems and his habit of registrating his body weight and dietary intake made possible a very detailed re construction of his dietary situation

His continuous decrease in body weight from January to December 1965 showed that he had been on an insufficient diet for a considerable time During this period he lost 33 kg in weight He was not obese at the beginning of the year During the juice diet period which included starvation for 16 days the total intake of calories was no doubt insufficient Furthermore the pro tein intake was very low never exceeding 30 g a day and included only vegetable proteins The very low urinary loss of nitrogen confirms that the protein intake was extremely low On the other hand the intake of minerals and vitamins appeared to be adequate The small amount of vegetable fat in the food probably included suf ficient amounts of essential fatty acids

At the beginning of the juice diet period the patient s body weight was 78 kg and at that time he was very lean and had almost no body stores of fat In spite of this he lost another 18 kg dur ing the following 143 days The weight loss dur ing the last part of this period was rather small (Fig 1) although his caloric intake was as low as 1600 calories per day It is known that starva tion reduces the metabolic rate more than would be expected from the reduction in body size (8) which results in an adaptation to the lowered food intake At the time of the investigation the patient was undoubtedly in an extremely protein depleted state This was quite obvious at the examination and was also shown by the very low urinary creatinine excretion It can be estimated that he had lost about half of his original total muscle mass In spite of this there were only moderate changes in the blood proteins The albumin and transferrin values were reduced and he also had slight anemia which did not appear to be due to iron deficiency

Protein starvation is reported to result in a gradual decrease in the essential amino acids in plasma and sometimes in a concomitant increase in the nonessential ones especially glycine and alanine both in children (26) and in adults (14) In the present subject no obvious decrease in the essential amino acids was observed but there were relatively high alanine and glycine levels It is possible that the relatively small changes in the plasma amino acid levels may be due to the fact that protein was continually released from th initially considerable protein depots in the muscles It is also possible that the subject who had lost body weight and had lowered his physical activity was adapted to the very low protein intake of about 30 g per day at least as far as the plasma amino acid balance is concerned Contrary to the observations in poorly nourished infants the taurine concentration in plasma was not increased and there was no excessive urinary excretion of taurine and beta aminoisobutyric acid in the present case (15)

The present individual showed very low cholesterol and phospholipid levels This is in ac cordance with previous observations in starving individuals (9 10) The triglyceride value was relatively higher although the intake of fat was very low and the fat depots extremely reduced This indicates a relatively high rate of triglyceride synthesis It is well known that a carbohydrate rich diet induces an increase in the triglyceride level to pathologically high levels in the plasma of certain individuals (cf 4) but this might imply not only a high relative but also a high absolute intake of carbohydrates

Earlier studies both in men (1) and in women (3 5) have shown that weight gain may be as sociated with an increase in the plasma triglyc eride level The present subject however did not show any higher level at the time of the third ex

amination when he had gained 25 kg in weight during a short period of time The weight increase in the present subject seemed to be caused mainly by a restitution of the muscle mass It seems reasonable to assume that such a weight gain should not influence the concentration of triglycerides in blood and other tissues in the same way as a weight gain due to the accumulation of fat

The low intake of sodium which was reflected by a very low urinary excretion of sodium (9 mEq/day at the first examination) might be the reason why the subject did not develop any signs of edema during the starvation period However when he returned to his ordinary vegetarian diet with a higher total sodium content transient edema was observed for a few days

His physical capacity was no doubt lowered during the last part of the juice diet period It is remarkable however that he increased in weight so soon and that he was able to fulfil very heavy work as early as a fortnight after he had finished the long starvation period This recovery occurred on a diet containing only vegetable protein

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THE PHYSICAL FITNESS OF OLD NORWEGIAN MEN AND WOMEN

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Abstract This study aimed at establishing the maximal level of aerobic power in old Norwegian people Twenty men and ten women above the age of seventy were physically tested

Two thirds of all the subjects had ECG changes at rest and/or during exerci e but none of them had to stop the testing procedure

Compared to young people the old subjects had a maximal oxygen uptake which was reduced by 50 Maximal heart rate as well as maximal oxygen pulse were considerably decreased indicating a reduced cardiac out put This gives reason to suggest that the old heart has an impaired functional performance

The respiratory efficiency is also decreased as indicated by the high ventilation equivalent

Although the subjects investigated do not represent a true random sample the result su gests that Norwegian people above seventy years of age possess an aerobic power or physical fitness which is only half of that at the age of 20–30 years

The maximal oxygen uptake is a basic measure of fitness for sustained and strenuous muscular work Many studies have shown that this parameter varies with age and sex For men peak values are usually found in the third decade of life From the age of thirty a gradual and steady decrease with age takes place Robinson (6) found that sedentary students and business men averaged 48 7 ml/min/kg body weight in their mid twenties which was reduced to 25 5 ml/min/kg in the seventies Strandell (7) found a similar average value of 24 5 ml/min/kg in 11 men between the ages of 70 and 83 Fischer et al (3) studied 20 men aged 70 to 80 years and found an average of 23 ml/min/kg

For women somewhat different age curves have been observed Astrand (8) reports an average of 39 9 ml/min/kg body weight among sedentary housewives in their twenties which was reduced to 28 4 ml/min/kg in the fifties Similar values have been found in female students and office workers studied by Hermansen (4 5) No data on maximal oxygen uptake are available for women above the age of seventy

This study was undertaken in order to obtain some information on the level of maximal oxygen uptake of old Norwegian men and women

MATERIAL

Twenty men and ten women above the age of seventy served as experimental subjects They were all recruited on a voluntary basis from old people s clubs in Oslo which have been organized by The Norwegian National Health Association s Institute of Gerontology Oslo It was a prerequisite that they could pedal a bicycle There is reason to believe that only the physically fittest part of the elderly population took part in these studies For instance most of the men took part once a week in gymnastic exercises which are one of the club activities These men and women do not represent a true random sample of the population but nevertheless the result may shed some light on the fitness level of old people

Some physical characteristics of the subjects are given in Table I

A clinical examination preceded the work tests and revealed degenerative disorders typical of old age (Table II) From a health point of view these men and women did not differ from the average population of comparable age Most of the subjects had typical complaints associated with the disorders According to the investigators judgement none of the disorders were so serious as to preclude participat on in the work tests The average aerobic work capacity of the d seased subjects did not differ from that of the other subjects

The clinical examination prior to the work tests included ECG with 12 leads and X ray of heart and chest in the erect position The heart volume was determined according to Jonsell and the upper normal limits were assessed at 540 ml/m for men and 500 ml/m for women The hemoglob n concentration total amount of hemoglobin sedimentation rate and serum cholesterol (Carr and Drekter s method) were examined The blood was taken in the postabsorptive state Blood volume was determined simultaneously by the dye d lution technique using Evan s blue

Table I *Physical characteristics of the subjects (mean ±S D and range)*

Subjects	No	Age (y)	Height (cm)	Weight (kg)	Sum of 10 skinfolds (mm)
Women	10	76 ± 3 4 72–84	157 ± 2 0 151–161	57 ± 4 8 44–70	114 ± 23 79–146
Men	20	76 ± 3 3 71–81	171 5 ± 6 2 161–183	71 ± 7 1 59–88	116 ± 30 72–172

The frequency of ECG changes at rest increases with age and is remarkably high in old people Abnormal ECG were found at rest in one of the ten women and in ten of the 20 men These changes may be accentuated durin, exercise Abnormalities occurred in another six of the women and three of the men during or after performance of muscular exercise (Table III)

Roentgenological determination of the size of the heart revealed slight enlargement in three men and two women all of whom suffered from defined diseases in the cardiovascular system (hypertension silent myocardial infarction and rheumatic valvular heart disease) The average heart size however is somewhat larger than at young ages (Table IV) This is in agreement with the findings of Fischer et al (3) and Strandell (7)

The mean value of blood volume hemoglobin concentration total amount of hemoglobin sedimentation rate and serum cholesterol are all within the normal ran e (Table IV)

Table II *Degenerative and other disorders discovered in the clinical examination of 20 old men and 10 old women*

	Male	Female
Cardiovascular disorders		
Angina pectoris	2	
Mb valvulosclerot cord		1
Mb cord hyperton	1	1
Mb cord rheumat	1	
Hypertonia arterial	2	1
ECG changes at rest including 1 male and 1 female suffering from silent heart infarction	10	1
Cerebral arteriosclerosis		
Dementia	1	
Vertigo paroxysm	3	
Disorders of locomotive apparatus		
Kyfoscoliosis and scoliosis	1	2
Coxarthrosis	3	
Other disorders		
Hernia ventralis	1	
Hydrocele testis	1	
Dupuytren s contraction	1	
Resectio ventr e ulcere	2	

Table III *Electrocardiographical findings in old men and women*

Subjects	No	Normal during rest and work	Pathological changes at rest[a]	Changes only during or after work test[a]
Women	10	3	1[b]	6
Men	20	7	10[b]	3

[a] ECG changes at rest and/or during exercise include ectopic beats rhythmic and conduction disorders abnormal T waves and ST segment variations

[b] Of the subjects with pathological ECG changes at rest six men and one woman showed additional changes during or after work tests

METHODS

Maximal oxygen uptake and related respiratory and circulatory functions were measured by having the subjects bicycle on an ergometer of the mechanical braking type Four submaximal work loads were performed each lasting 5–8 minutes The measurements were taken in the last minutes of the exercise periods At least two maximal work loads were performed each lasting approximately three minutes and the measurements were taken in the last minute of the period The highest value was considered as the true maximal uptake Only one maximal work load was performed on the same day

The linear relationship between oxygen uptake and work output at submaximal loads was established and the work level was found at which a further increase in work rate did not bring about an increase of oxygen uptake This levelling off of oxygen uptake was used as a criterion that the maximal value of oxygen uptake was reached This criterion was not successfully achieved in four men and three women However the RQ value in all cases exceeded 1 0 indicating exercise strain close to maximum

Respiratory measurements were taken by using an open circuit system Expired air was usually collected into a balanced tank and occasionally in Douglas bags Samples of gas were withdrawn for analysis by means of the ml Scholander method

Heart rates were measured from electrocardiograms recorded during the last half minute of the working period

RESULTS

Oxygen uptake at submaximal work

Oxygen uptake measured at the steady state" level of submaximal work loads increased linearly with the rate of work The oxygen cost of bicycling appeared to be somewhat greater for men than for women (Fig 1)

Table IV *Si e of the heart and blood constituents* (*mean* ±S D *and range*)

Subjects	No	Heart vol. (ml)	Blood vol (ml)	Hemoglobin (g/100 ml)	Total Hb (g)	ESR (mm/h)	Serum cholesterol (mg/100ml)
Women	10	635 ±126 480–880	4 0 ±0 45 3 4–4 6	14 0 ±0 86 12 5–15 2	555 ±73 450–650	12 ±8 0 2–28	[illegible]84 ±54 212–377
Men	[illegible]0	813 ±132 550–1090	5 9 ±0 53 4 9–6 8	14 7 ±0 84 12 7–16 4	858 ±96 70–1102	6 ±4 5 1 19	[illegible]70 ±48 174–340

The oxygen uptake/work output relationship is for men different from that of young subjects inasmuch as oxygen uptake is somewhat higher at light work loads This difference is not demon strated in women

The figure shows how well the levelling-off of

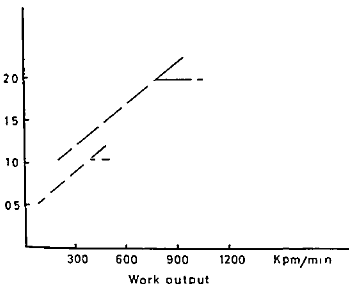


Fig 1 Average oxygen uptake in 0 old men and 10 old women during bicycling at different loads ● male × female

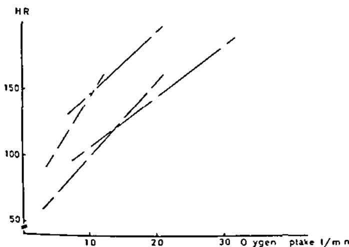


Fig Heart rate oxygen uptake relation ship in old men and women compared with young people in estigated in the same laboratory (5) ● men 70–84 y male students □ women 70–84 y ■ female students

Table V *Maximal oxygen uptake heart rate and pulmonary ventilation (mean ±S D and range)*

Subjects	No	Maximal O_2 uptake l min	Maximal O_2 uptake ml min/kg	Maximal O_2 pulse	Highest recorded H R.	Highest pulm vent (l/min BTPS)
Women	10	1 09 ±0 14 0 81–1 [illegible]	19 ±2 5 16–24	7 4 ±1 0 5.2–8 2	153 ±13 137–172	35 ±4 5 29–42
Men	20	1 90 ±0 28 1 51–2 49	27 ±3 1 2[illegible]–33	12 7 ±1 8 9 5–17 4	150 ±15 118–176	70 ±13 1 46–93

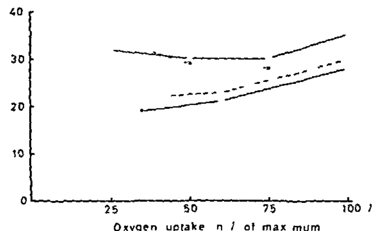


Fig 3 Ventilation/oxygen uptake relationship in old men and women compared with younger people investigated in the same laboratory (4) ● male 70–84 y O male 30–39 y female 70–84 y ∧ female 30–39 y

oxygen uptake at its maximal value was achieved The data in general may be interpreted as an indication of conserved efficiency in bicycling at old ages

Maximal oxygen uptake

Maximal oxygen uptake is given in Table V The mean value for men is 1 90 l/min or 27 ml/min/kg body weight Similar data for women are considerably lower 1 09 l/min or 19 ml/min/kg body weight.

Circulatory response to exercise

Heart rates recorded at the end of the exercise periods are linearly related to the uptake of oxygen which is a well established finding (Fig 2) Men have a lower heart rate at the same metabolic level (Table VI) The highest recorded heart rate which is considered to represent the maximal value averaged about the same for men and women but maximal oxygen pulse was definitely lower for the women (Table V) Maximal heart rate in these old subjects was markedly reduced in relation to young people

Table VI *Heart rate at various levels of oxygen uptakes work rates and pulmonary ventilations (mean ±S D)*

Subject	No	O_2 uptake l min STPD 0 5	O_2 uptake l min STPD 1 0	O_2 uptake l min STPD 1 5	Work rate kpm min 150	Work rate kpm min 300	Work rate kpm min 600	Pulm vent l min BTPS 15	Pulm vent l min BTPS 25	Pulm vent l min BTPS 50
Women	10	103 ±10 6	141 ±11 8	—	115 ±12 1	135 ±10 9	—	104 ±11 9	132 ±12 6	—
Men	20	70 ±14 5	99 ±13 1	127 ±14 7	96 ±18 1	110 ±17 1	136 ±16 1	80 ±15 8	93 ±17 7	1[illegible]9 ±18 9

Respiratory response to exercise

The ventilation equivalent has been calculated (ventilation rate divided by oxygen uptake) at various levels of oxygen uptake The data are presented in Fig 3 The lower respiratory efficiency which comes with increasing age is clearly demonstrated in the figure

Contrary to the conditions in youth the women in this investigation had the most efficient ventilation At light work rates both men and women have hyperventilation At the highest metabolic rate the ventilation equivalent in these old subjects exceeds that of young ages The highest recorded pulmonary ventilation however is lower than in young people (Table V) This decrease follows the decrease in aerobic power (8) The ventilation values in men observed in this investigation are higher than noted in previous publications (3 6) The highest recorded ventilation was double that in old women There were however great interindividual variations

DISCUSSION

At rest 40 / of the subjects had pathological ECG During or after exercise abnormal ECG were found in an additional 20 / so that altogether abnormal ECG were recorded in about /3 of all the subjects This figure corresponds well to data reported by Åstrand (9) who studied men between the ages of 55 and 70 years and found abnormal ECG in 50% of the subjects using a similar testing procedure Similar data for men 70–83 years are reported by Strandell (7) These findings indicate pathological conditions in the myocardium or an insufficient coronary blood flow during exercise Despite the signs of impaired cardiac function none of our old subjects complained of angina pectoris during the work test and the investigators found no indication to stop the test at submaximal work intensities

From the ventilation/oxygen uptake relationship it is seen that especially the old men had a fairly well preserved bellows function of the lungs The pulmonary ventilation efficiency on the other hand is in old subjects (2) inferior to that found in young persons (Fig 3) The ventilation equivalent is higher in senescents particularly in males The reduced efficiency may be due to the aging process in the lungs resulting in emphysema In contrast to these observations Åstrand (8) did not find any change with age in the ventilation/oxygen uptake relationship in females

The oxygen cost of work at submaximal levels was found to be somewhat greater for old men than for old women A similar sex difference has been found at younger ages (5 6) but the reason for this difference cannot be stated

This study revealed a somewhat lower work efficiency in bicycle riding in old compared to young men For instance at a work rate of 300 kpm/min the oxygen consumption was on average 1200 ml/min in the old subjects compared to 1030 ml/min for young men tested by the same method (5) This is a small but significant difference A similar difference in work efficiency between young and old subjects has been observed by Åstrand (8) and Fischer et al (3) but not by Strandell (7) As suggested in an earlier publication (2) the reason is probably due to increased stiffness and reduced elasticity of the supporting joint tissue and to some extent also to impaired coordination of the movements This difference in work efficiency is however not demonstrated in the women in this investigation

The maximal oxygen uptake of the old men averaged 27 ml/min/kg body weight which is in close agreement with average values reported by other investigators (3 6 7) Norwegian males in their twenties engaged in sedentary occupations average 48 ml/min/kg body weight (1) At ages above 70 years the maximal aerobic power is thus reduced by about 50

Sedentary Norwegian women in their twenties average 36 ml/min/kg body weight and the women above the age of 70 years 19 ml/min/kg body weight The relative decline in maximal aerobic power thus seems to be approximately the same in the two sexes Regarding old females no available comparable investigation has been found in the literature

Heart rate responded to progressively increasing work loads in the same linear relation to oxygen uptake as has been established in numerous studies of young and middle aged subjects However the slope of rectilinear curve is steeper in the old subjects as is apparent from Fig 2 Assuming the same cardiac output for a certain oxygen uptake in young as in old subjects these deviating curves indicate that in old people either

stroke volume or A–V difference or both do not present the same response to increasing work load As shown by Strandell (7) the arterial oxygen saturation is unchanged with age Consequently the low oxygen pulse is probably related to a decreased stroke volume

The decrease in maximal heart rate and stroke volume with age bring about a lower maximal cardiac output This cannot be fully compensated by a higher arterio venous oxygen difference which is observed in old subjects (7) Since cardiac output was not measured in our investigation it is not possible to confirm these observations

The lower stroke volume and the increased heart size indicate an increased residual blood volume within the heart From a physiological point of view there is reason to infer that the inotropic effect and the contractility of the heart muscle are reduced Thus the aged heart may have a decreased pumping capacity with an impaired functional performance

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BLOOD FLOW AND UPTAKE OF OXYGEN AND SUBSTRATES IN FOREARM MUSCLE AND SUBCUTANEOUS FAT TISSUE IN MAN

A Study in Normal and Diabetic Subjects

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Abstract Blood flow and arteriovenous differences in oxygen, glucose lactate pyruvate free fatty acids glycerol, acetoacetate and β hydroxybutyrate were measured in forearm muscle tissue before during and after insulin infusion in healthy and diabetic subjects The same metabolic data were collected for forearm subcutaneous fat tissue

Significant correlations were obtained between the flow and the oxygen uptake in the two tissues both before and after insulin On the other hand there were no correlations between the flow and the uptake of any single substrate or the total net uptake of the substrates Concerning the arterial levels of the substrates there was a significant correlation between that of free fatty acids in the diabetics and the muscle blood flow

In an earlier publication we studied the blood flow and the uptake of oxygen and substrates in vivo in human skeletal muscle and subcutaneous fat tissue in one healthy and one diabetic subject (4) That paper dealt in detail with the net tissue uptake of oxygen glucose lactate pyruvate free fatty acids (FFA) glycerol and β hydroxybutyrate Similar studies on an extended patient material are in progress The present report deals with the relationships between the blood flow of forearm muscle and subcutaneous fat tissue and the uptake of oxygen and substrates in these tissues

MATERIAL AND METHODS

Ten individuals seven diabetics and three healthy controls, were studied Clinical data for the diabetics are given in Table I The healthy controls were males 3–35 years of age

The investigations were performed in the morning when the subjects had been fasting overnight The diabetic subjects had not taken any insulin for the last ?4 hours

The methods used were mainly as in the previous study (4) However one of the catheters was inserted into a superficial forearm vein on the ulnar side with its tip placed about 5 cm distal to the elbow This vein was considered to drain mainly superficial forearm tissue i e subcutaneous fat tissue and skin When the subjects were performing muscular work involving the forearm muscle no decrease in the venous oxygen saturation was observed in blood samples from the superficial vein catheter whereas those from the deep vein catheter decreased to values below 25 oxygen saturation The elimination curves of the locally injected radioactive xenon (Xe^{133}) saline solution were continuously recorded throughout the experiment from both the muscle and the fat tissue by means of two identical scintillation crystal detectors of light weight (Dansk Impulsefysik)

Crystalline insulin (Vitrum®) crystallized twice and claimed to be free of glucagon was given by continuous infusion altogether 0 ?–0 8 I U Two of the controls received 0 4 I U of insulin and the third one 0 8 I U The amounts of insulin administered to the diabetics are given in Table I The insulin was dissolved in ?0 ml of saline and infused during 20 minutes into the catheter in the brachial artery of the forearm studied During the infusion the arterial blood samples were drawn from a catheter in the contralateral brachial artery Three blood samples were taken at five minute intervals before the insulin administration Blood sampling was repeated 5 ?0 30 and 60 minutes after the start of the infusion The chemical analyzes were extended to include also acetoacetate (8) The oxygen equivalent values (μl oxygen/μmoles substrate) used were for glucose 135 lactate 67 pyruvate 56 FFA 515 glycerol 78 acetoacetate 87 and β hydroxybutyrate 101

RESULTS

Muscle tissue

Fig 1 shows the general metabolic situation in the muscle tissue before and 20 minutes after the start of the infusion with respect to the total

the variations in oxygen uptake or whether it is the variations in the metabolic need for oxygen in the tissue that influence the blood flow It is well known that the skeletal muscle blood flow increases during exercise when the oxygen uptake also increases (5) for a review see Carlsten and Grimby (2) For the myocardial tissue a relationship similar to that in skeletal muscle has been observed (1 3) Such a relationship between blood flow and oxygen uptake does not exist in the brain tissue where the oxygen consumption is kept constant in spite of great variations in the blood flow for a review see Lassen (6) In the exercising muscle it seems reasonable to assume that the augmented need for oxygen is the main factor which regulates the blood flow In the resting muscle on the other hand it could be assumed that the oxygen uptake should be relatively constant despite variations in flow

Such a constancy of the muscle oxygen uptake was observed by Stainsby and Otis (7) when they experimentally changed the blood flow by removing the neurogenic vasoconstrictor tone of the muscular blood vessels In their studies of the relationship between blood flow and oxygen uptake the initial control values showed however fairly constant arteriovenous oxygen differences resulting in a correlation between the blood flow and the oxygen uptake Therefore the observations of Stainsby and Otis (7) can be taken to show that it is the metabolic demand for oxygen which regulates also the blood flow of the resting skeletal muscle The present observation of great differences in oxygen uptake between different individuals can thus be attributed to differences in the need for oxygen or energy supply This view is supported by the observation of a relationship between the blood flow and the arterial levels of FFA and ketone bodies in the diabetics in whom these substrates were the main energy sources

For the fat tissue the available metabolic data do not allow any conclusions or proposals as to the mechanism of the regulation of the oxygen uptake and blood flow The main task of the adipose tissue is to store energy The net uptake of substrates is therefore not adapted merely to the energy need of the fat tissue per se as in the muscle tissue

ACKNOWLEDGEMENT

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ACCUMULATION OF CHLORPROPAMIDE CAUSED BY DICOUMAROL

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Abstract A description is given of a patient who had received 375 mg chlorpropamide daily for two years for Parkinsonism. Three months after the institution of anticoagulant therapy with dicoumarol he had a hypoglycaemic attack The chlorpropamide concentration in the serum was remarkably high. Clinical experiments revealed that the administration of dicoumarol to patients who are receiving chlorpropamide leads to accumulation of the latter The half-life of chlorpropamide in the blood is increased from the normal value of about 40 hours to about 90 hours during treatment with dicoumarol.

Hypoglycaemic attacks rarely occur during treatment with chlorpropamide in therapeutic doses However during the past few years there have been a number of reports of such attacks (4 7 17 20 21 23 25 26) Accumulation of chlorpropamide in the body has been put forward as a possible trigger mechanism in such hypoglycaemia, but in the majority of the reports mentioned the concentration of chlorpropamide in the serum has not been investigated.

We have previously drawn attention to the fact that the administration of sulphaphenazole and phenylbutazone (6) and dicoumarol (19) simultaneously with tolbutamide may lead to accumulation of the latter with a risk of the development of hypoglycaemia. It has similarly been demonstrated that the combination of chlorpropamide and phenylbutazone may trigger off hypoglycaemic attacks (8) and that pyrazole derivatives may potentiate the effect of carbutamide (16)

In 1960 Gates and Hyman (9) suggested the use of tolbutamide in the treatment of Parkinsonism, and in the same year Gillhesby and Paton (10) published some promising results of chlorpropamide therapy in this disorder However later investigations of tolbutamide (11 14) and chlorpropamide (2) therapy in Parkinsonism have not confirmed these favourable results.

The observation of hypoglycaemic symptoms in a patient with Parkinsonism who was receiving treatment with both chlorpropamide and dicoumarol drew our attention to the possibility of an interaction between these two drugs

CASE HISTORY

A 67 year-old man with familial disposition to Parkinsonism had no significant illness in his case history During the past ten years he became increasingly troubled by tremor of both hands and of his head In the course of time he had tried a variety of anti Parkinson drugs, without any particular benefit. In 1961 operation was contemplated, but not undertaken and instead he was given 375 mg chlorpropamide daily In July 1963 he was admitted with deep phlebitis in the right leg complicated by an infarct of the lung, and anticoagulant therapy with dicoumarol was therefore instituted. On the morning of 22.x 63 he was admitted for the first time to this department of medicine with a diagnosis of cerebral haemorrhage During the morning of the day preceding that of admission the patient felt unwell with difficulty in speaking; the symptoms disappeared after he ate his breakfast. During the hours immediately preceding admission he had once again become increasingly apathetic and had difficulty in speaking.

On admission he was found to be apathetic and drowsy with incomprehensible speech. There was a Babinski reflex on the right side but no tremor or rigidity The blood sugar was 40 mg per 100 ml, and he woke immediately after intravenous injection of glucose his speech returned to normal, and there was now tremor and rigidity of both arms Chlorpropamide was withdrawn. The following day his blood sugar lay in the range 60–80 mg per 100 ml (Hagedorn–Normann Jensen method) The usual blood investigations were normal in particular there was nothing abnormal in the liver function tests or serum creatinine. The prothrombin-proconvertin value was, however 77% due to the dicoumarol therapy Six hours after admission and 27 hours after the last dose the chlorpropamide concentration was found to be 18 4 mg per 100 ml. During the following days there was a slow fall in concentration, showing a half-life of 80–90 hours, in contrast to the normal half-life of 3 -38 hours (18)

The initial chlorpropamide concentration was high as according to Carlozzi et al (5) a serum concentration of approx. 12 mg per 100 ml is to be expected after long term administration of 375 mg chlorpropamide daily This fact in association with the increased half life suggested that the accumulation of chlorpropamide was due to dicoumarol The dicoumarol was therefore gradually withdrawn and six weeks after withdrawal the determination of the half life of chlorpropamide was repeated this was now found to be normal with a value of 30 hours On the basis of these observations we have carried out some clinical investigations in order to elucidate whether dicoumarol affects the excretion of chlorpropamide or not

CLINICAL EXPERIMENTS

Methods

The method used for the determination of chlorpropamide in the blood was that described by Spingler (25) for the determination of tolbutamide The presence of dicoumarol did not interfere with the analysis

Chlorpropamide in the urine was determined by a modification of the method for the determination of carboxytolbutamide in urine (4)

S 35 labelled chlorpropamide was determined as the total activity in the plasma

Thin layer chromatography of serum and urine for the identification of chlorpropamide and any possible metabolites was carried out according to the method described for tolbutamide (19) In this system the Rf value for chlorpropamide is 0 7

The dicoumarol in the serum was determined by means of the method described by Axelrod et al (1)

RESULTS

Three diabetic patients who had been receiving treatment with chlorpropamide for at least two weeks at a dosage of 250 mg daily were given dicoumarol in such doses as to achieve a prothrombin proconvertin value of 40 % The fasting chlorpropamide concentration in the plasma was determined at intervals of a few days 3–4 days after the beginning of the administration of dicoumarol there was a clear increase in the plasma chlorpropamide concentration from an average of 7 6 mg per 100 ml to 13 3 per 100 ml (Fig 1) in all three patients Measurements were made on one of the patients for several weeks after the withdrawal and it can be seen that the chlorpropamide concentration did not return to predicoumarol levels until about 17 days after the withdrawal of dicoumarol Chromatography of samples of serum revealed that these contained only chlorpropamide

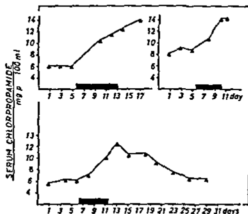


Fig 1 Serum chlorpropamide concentration in three patients before during and after dicoumarol treatment. ■ indicates periods of dicoumarol treatment

Two non-diabetic patients were given 450 mg chlorpropamide labelled with 20 μc S^{35} i v before and after six days of treatment with dicoumarol (35-S labelled chlorpropamide was placed at our disposal by Chas Pfizer and Co Inc New York) The concentration of chlorpropamide in the plasma was followed for 72 hours after each injection Before treatment with dicoumarol the half life of chlorpropamide was found to be 38 and 35 hours in the two patients whilst during dicoumarol these values were found to be 94 and 84 hours respectively At this time the dicoumarol concentrations in the serum were 29 and 24 5 μg per ml respectively

Fig 2 shows the fall in concentration of chlorpropamide before and during dicoumarol treatment in one of these patients there was a simultaneous fall in the urinary excretion from 330 mg in the 72 hours before dicoumarol to 180 mg in the 72 hours during dicoumarol treatment Chromatography of the urine revealed no radio-

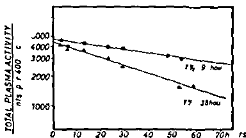


Fig 2 Serum chlorpropamide concentration in one patient after 450 mg S-35 labelled chlorpropamide i v ▲ before dicoumarol ● during dicoumarol treatment.

activity other than that corresponding to the Rf value of chlorpropamide There was no definite difference in the distribution volume of chlor propamide before and during dicoumarol therapy in these two patients

DISCUSSION

According to Johnson et al (15) at least 85–90° of the chlorpropamide is excreted un changed in the urine within the first three days of an intravenous injection the half life in the blood is 32 to 36 hours After the administration of dicoumarol there was clear increase in the chlorpropamide concentration in the plasma in three patients from an average of 7 6 mg per 100 ml to an average of 13 3 mg per 100 ml the increase was observed about 2–3 days after the beginning of the dicoumarol treatment This is due to the fact that the half life in the blood was doubled from about 40 hours to about 90 hours As no definite difference was found in the distribution volume of chlorpropamide before and during the administration of dicoumarol in our two patients it must be assumed that di coumarol primarily reduces the renal clearance of chlorpropamide

In two earlier reports (12 19) we have pointed out the fact that dicoumarol may have other ef fects on the organism than the known and clini cally applied inhibition of prothrombin synthesis in the liver In these papers we demonstrated the probability that dicoumarol inhibits the drug metabolizing systems in the liver as the ad ministration of dicoumarol to patients receiving treatment with tolbutamide or diphenylhydantoin leads to accumulation of these two compounds the rate of excretion of which is largely deter mined by enzymatic oxidation in the liver In addition it has been shown that dicoumarol has a uricosuric effect (13) possibly because of inhibi tion of resorption of uric acid in the renal tubules

In diabetics the high concentration of chlor propamide in the plasma is presumably not al ways sufficient to produce hypoglycaemia as Beaser (3) has been able to produce such toxic effects of chlorpropamide as dizziness muscular weakness in the legs paraesthesia and in one case ataxia in chlorpropamide sensitive diabetics without any simultaneous development of hypo glycaemia

In certain patients complicating factors which reduce the carbohydrate depots are presumably necessary for the development of hypoglycaemia The patient with Parkinsonism described here had thus received a combination of chlorpropamide and dicoumarol for three months before he devel oped hypoglycaemic attacks From the results of our clinical experiments it must be assumed that the serum chlorpropamide concentration has been high throughout the three months and it is con ceivable that a slightly reduced consumption of carbohydrate during the days preceding the attack was the factor which triggered off the hypogly caemic attack It is similarly worth noting that the attacks occurred in the early morning In the three diabetic subjects there was a fall in blood sugar con centration during treatment with dicoumarol but it is not possible to assess these values in more detail partly because two of the patients had re ceived chlorpropamide for only 2–3 weeks and partly because there were wide fluctuations in the blood sugar levels in the third subject through out the period of the investigation presumably due to uncontrolled consumption of carbohydrate

As far as is known dicoumarol has no inherent hypoglycaemic effect When dicoumarol is ad ministered to diabetics receiving therapy with tolbutamide or chlorpropamide it is important to anticipate either possible toxic side effects such as muscle weakness dizziness and par aesthesia none of which however were observed in our patient or hypoglycaemia despite the fact that the antidiabetic drugs are administered only in small therapeutic doses Current investigations suggest that sulphaphenazole may also cause ac cumulation of chlorpropamide

ACKNOWLEDGEMENT

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PROPRANOLOL (INDERAL) IN THE LONG TERM PROPHYLAXIS OF VENTRICULAR ARRHYTHMIAS

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Abst act The results of prophylactic treatment for more than 2 / years with propranolol in four patients with attacks of ventricular tachyarrhythmia are reported Two of the patients are children

Good results were obtained in three of the patients who lead a satisfactory life with no or rare attacks of tachycardia In one patient the treatment has only de creased the frequency of the *severe* attacks somewhat.

The growth and development of the two children have been normal In one patient more pronounced brady cardia with drowsiness has been encountered as well as symptomless thrombocytopenia In another patient the medical treatment had to be supplemented by a pace maker

The adrenergic beta receptor antagonist pro pranolol (Inderal) has gained widespread use dur ing recent years in the treatment and in the prophylaxis of cardiac arrhythmias as well as in an increasing number of other conditions

The predecessor of propranolol pronethalol produced sarcomas in mice Propranolol itself may cause dangerous complications such as heart failure and arrhythmias (2 6 8 11) and episodes of hypoglycaemia have recently been reported (1 5) Consequently careful observation of patients who receive the drug is necessary especially in long term treatment.

In this paper our experience with the long term prophylactic use of propranolol in ventricular arrhythmias in four patients is described both as regards the ultimate effect and the observation of side-effects Two children have been treated for 31 months while the treatment in two adult patients has lasted for 29 months and 28 months respectively The events during the first 1–1 ¹/₂ years of treatment have been published previously (10 11) The emphasis in the case reports below is therefore laid on the events during the contin ued treatment.

CASE REPORTS

Case 1

Girl born 1956 had for some years suffered from Adams–Stokes attacks due to runs of ventricular extrasystoles on exertion Treatment with propranolol 10 mg four times daily was instituted in October 1964 the effect of the drug bein, tested on the exercise electrocardiogram (10) She had no attacks during the treatment. In December 1966 the treatment was stopped and two weeks later she had a severe attack while running in stairs The treatment was promptly resumed in the same dosage Half a year later she had another attack during vigorous exercise and the dosage was increased to 15 mg four times daily

Growth and development have been normal and no side-effects have been noted

Case 2

Girl born 195., started having Adams–Stokes syncopes after a severe attack of measles At rest she had per sistent bradycardia (about 40/min) but on exertion she had runs of multifocal ventricular extrasystoles

After an initial trial with different drugs, the effect of which were tested on the exercise electrocardiogram (10) treatment with propranolol was started in October 1964

The initial dosage of 80 mg daily was gradually re duced to 30 mg daily during the first 13 months be cause of development of drowsiness when a constant dose was given The drowsiness always disappeared completely within 24 hours after withdrawal of the drug Because of this cumulating effect *intermittent* treatment has been given since February 1966 first with 20 mg three times daily except on Sundays, and from May 1967 30 mg three times daily except on Sundays On this intermittent treatment no more episodes of drowsiness were en countered

In spite of the treatment she continued to have attacks *Before* the treatment she had an attack from once every -3 weeks to once in half a year *During* the treatment the intervals have in general been about the same though longer periods, 4–6 months at least, have elapsed between the more *severe* and long lasting attacks On two oc casions the treatment was stopped for a short time but each time she then had daily attacks—the last time for ten days—until the treatment was resumed

In this patient transient thrombocytopenia was noticed during two periods with the lowest values of 49 000/μl and 79 000/μl respectively No bleeding tendency was seen and the thrombocytopenia subsided during the continued treatment. No other side-effects apart from the mentioned drowsiness were encountered

Growth and development have been normal In February 1967 she started to menstruate regularly

Case 3

Female born 1916 had increasingly severe attacks of ventricular tachycardia since 1962 In between there were episodes of dizziness, probably due to sinoauricular block The attacks of tachycardia became more frequent.

Treatment with propranolol was initiated in December 1964 and the attacks of tachycardia subsided for a long time—until March 1966 However the periods with sinoauricular block accompanied by spells of dizziness became more frequent, in spite of gradual reduction of the dosage of propranolol from 30 mg four times daily to 10 mg twice daily

In March 1966 the attacks of tachycardia reappeared, alternating with sinoauricular block or extreme bradycardia accompanied by spells of dizziness or syncope The condition remained unchanged in spite of discontinuation of propranolol for one month

As the situation was precarious a pacemaker was implanted This prevented the episodes of bradycardia, but the attacks of ventricular tachycardia persisted and necessitated resumption of the treatment with propranolol 30 mg four times daily

During this *combined* treatment with a pacemaker and with propranolol there have been no further episodes of bradycardia and no further attacks of tachycardia, and the patient s exercise tolerance has been fairly good (7)

Case 4

Male born 1916 had increasingly frequent attacks of ventricular tachycardia for four years when treatment with propranolol was started in January 1965

After the start of the treatment he had no attacks for 2½ months However the attacks then reappeared—though infrequently—necessitating a gradual increase in dosage from 60 mg to 120 mg daily during the first year of treatment. The acute attacks of tachycardia had all to be stopped by DC countershock.

In the summer of 1966 an increasing number of attacks occurred until the dosage was increased to 240 mg daily in September 1966 After a minor attack in October 1966 and another in November 1966 there have been no further attacks, and in April 1967 the dosage was reduced to 200 mg daily

During the initial treatment he complained of dizziness tiredness thirst and a sensation of cold, but these symptoms disappeared when the treatment was continued

DISCUSSION

Little information is available regarding the long term prophylactic use of propranolol in ventricular tachyarrhythmias

Harris (4) reported on a patient with a four week history of multiple Adams Stokes attacks due to recurrent episodes of ventricular tachycardia Treatment with quinidine or procainamide had no effect Within a few hours of the first oral dose of 20 mg of propranolol the attacks of ventricular tachycardia stopped and the patient remained free of attacks for 14 months on a dosage of 60 mg of propranolol daily

Gettes and Surawicz (3) treated life threatening paroxysmal ventricular tachycardia in two patients with propranolol thereby suppressing the arrhythmia for as long as 21 months Other antiarrhythmic agents were ineffective or poorly tolerated

Ward s report (9) seems to be the only one concerning treatment of arrhythmias in *children* with a beta receptor blocking agent and there have been no reports on long term treatment in children

With the long term treatment in our small material a really good effect was obtained in three of the patients (cases 1 3 and 4) They lead a satisfactory life with no or very rare attacks of tachycardia In one of these patients (case 3) the medical treatment had to be supplemented by a pacemaker which prevented Adams Stokes attacks due to sinoauricular block or bradycardia. In the last patient (case 2) the effect of the treatment is less satisfactory However the frequency of her severe attacks has decreased

No impairment of the growth and development of the two children was noted and the older girl is menstruating regularly

The side-effects have been drowsiness and pronounced bradycardia in one patient (case 2) which necessitated intermittent treatment In another patient (case 3) with periods of sinoauricular block, these periods became more pronounced during the treatment. Furthermore symptomless thrombocytopenia was encountered in one patient (case 2)

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THE EFFECT OF EXERCISE ON THE SIZE OF THE SHUNT IN PATIENTS WITH ATRIAL SEPTAL DEFECTS

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Abstract The pulmonary and systemic flow was measured at rest and during exercise in 13 patients with atrial septal defects, of whom 12 had a normal pulmonary arterial pressure The pulmonary flow ratio decreased in all the patients and the decrease was clearly dependent upon the value at rest The pulmonary blood flow increased during exercise This increase was greatest in the patients with a small blood flow in the pulmonary artery and smallest in those with the largest pulmonary blood flow at rest The shunt decreased correspondingly inasmuch as the systemic blood flow was normal both at rest and during exercise All the changes tend to produce a normalization of the haemodynamic conditions during exercise in patients with uncomplicated atrial septal defects and explain why the working capacity of these patients is independent of the magnitude of the shunt at rest

It has already been shown that the working capacity and symptoms in patients with uncomplicated atrial septal defects (ASD) are not related to the magnitude of the shunt (1 4 6 7) This paper is concerned with the effect of exercise on the relative and absolute size of the shunt in these patients particularly as earlier investigations have shown conflicting results (5 8 11)

MATERIAL AND METHODS

Thirteen patients with ASD (six men and seven women) with ages ranging from seven to 46 years were studied by right sided heart catheterization All patients showed an increase in the oxygen saturation of the blood of at least 5% from the venae cavae to the right atrium and the defect was crossed in every case Two patients (nos 5 and 11) had ostium primum defects and the remainder defects of the ostium secundum type One patient (no 4) had been operated upon one year earlier for pulmonary stenosis and this had produced a fall in the systolic pressure in the right ventricle from 80 to 32 mm Hg Three catheters were used during the examination an Ödman catheter in the femoral artery a Lehman USCI catheter from the basilic vein to the inferior vena cava (IVC) and a Lehman USCI catheter from the femoral vein to the pulmonary artery (10) In the earlier patients the catheter in the IVC had an end hole and in the remainder side holes and was drawn backwards into the superior vena cava (SVC) during exercise The exercise lasted 6 minutes and was carried out in supine position using a bicycle ergometer with loads varying from 150 to 400 kpm/min

In three patients (nos 1 8 and 10) the exercise consisted of lifting their legs at a rate of 72 times/min The oxygen saturation of the blood was measured both at rest and during exercise on samples from the femoral artery pulmonary artery IVC and SVC using a haemoreflector (Elema–Schonander) The expirated air was collected for 3–5 min in a Douglas bag the content of which was analysed on a Lloyd gas analyser and the volume measured on a gas meter The output of the right ventricle was calculated according to the Fick principle The pulmonary flow ratio (PFR = pulmonary blood flow/systemic blood flow) was calculated both at rest and during exercise by the following formula (3)

$$PFR = \frac{O_2 \text{ satur \% of pulm veins} - O_2 \text{ satur \% of caval veins}}{O_2 \text{ satur \% of pulm veins} - O_2 \text{ satur \% of pulm art}}$$

The average oxygen saturation in the IVC and SVC was taken as the O_2 saturation % of the caval veins while at rest During exercise 75% of the systemic blood returns via the IVC (10) so the formula

$$\frac{(O_2 \text{ satur \% of IVC} \cdot 3) + O_2 \text{ satur \% of SVC}}{4}$$

was used to derive the figure for the O_2 saturation of the caval blood The systemic blood flow was calculated as the pulmonary blood flow/PFR and the shunt as the difference between the pulmonary flow and the systemic flow

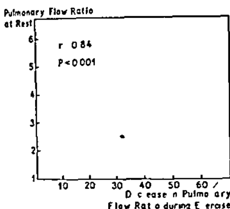
RESULTS

The cardiac pressures can be seen in Table I In all 13 patients normal pulmonary vascular resistance was found varying from 33 to 122 dyn sec cm^{-5} (mean 71 S D 23) None of the patients had signs of a right to-left shunt as the

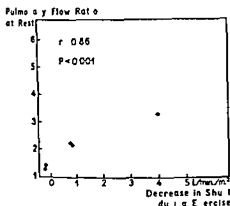
Table 1 *Haemodynamic data in 13 patients with atrial septal defect at rest and during exercise*

Case no sex	Age BSA (m^2)	O_2 consumption (ml/min)	O_2 capacity (vol)	O_2 saturation Fem art	Pulm art	SVC	IVC	Pulm blood flow (l/min)	($l/min/m^2$)	Syst blood flow (l/min)	($l/min/m^2$)	Pulm flow ratio	Shunt (l/min)	($l/min/m^2$)	Pressures (mm Hg) Pulm art (s d)	Right ventr (s d)	PVR (dyn sec cm^{-5})
1	22	R 270	19 8	93	89	77	73	21 7	11 7	6 6	3 6	3 3	15 1	8 1	25/11	31/5	33
♂	1 85	E 644			81	75	73	21 0	12 4	15 3	8 3	1 5	7 7	4 1	26/16		
2	23	R 278	16 9	97	75	65	69	7 5	3 6	5 4	2 6	1 4	2 1	1 0	27/12	32/9	75
♀	2 11	E 872			60	61	48	13 8	6 6	11 1	5 3	1 2	2 7	1 3	35/17		
3	12	R 193	17 5	98	84	73	73	8 0	7 2	4 5	4 1	1 8	3 5	3 1	28/10	34/5	100
	1 11	E 486			72	75	56	10 8	9 7	7 7	7 0	1 4	3 1	2 7	34/13		
4	19	R 207	18 7	98	94	70	74	29 6	20 1	4 6	3 1	6 5	25 0	17 0	35/12	44/2	50
♀	1 47	E 910			80	71	50	28 2	19 1	11 8	8 0	2 4	16 4	11 1	32/10		
5	23	R 251	21 6	97	88	77	77	12 6	6 1	5 7	2 8	2 2	6 9	3 3	24/12	31/4	76
♂	2 08	E 1043			70	74	54	17 7	8 5	12 6	6 0	1 4	5 1	2 5	32/13		
6	10	R 154	17 7	96	84	73	79	7 4	6 4	4 4	3 8	1 7	3 0	2 6	22/10	22/5	76
♀	1 15	E 574			62	64	53	9 6	8 3	8 1	7 0	1 2	1 5	1 3	26/10		
7	33	R 245	19 3	97	85	71	79	10 7	6 5	5 9	3 6	1 8	4 8	2 9	21/9	29/4	60
♂	1 65	E 974			65	73	47	15 7	9 6	12 1	7 3	1 3	3 6	2 3	28/16		
8	7	R 160	16 4	98	90	75	81	12 3	13 4	4 9	5 3	2 5	7 4	8 1	29/11	33/4	65
♂	0 92	E 320[a]			82	70	71	12 3	13 4	7 2	7 9	1 7	5 1	5 5	25/12		
9	46	R 227	19 5	97	88	70	76	13 4	7 4	5 0	2 8	2 7	8 6	4 8	41/18	41/4	122
♂	1 80	E 1090			71	67	51	21 4	11 9	13 3	7 4	1 6	8 1	4 5	72/28		
10	10	R 195	19 3	98	88	73	64	10 3	9 5	1 4	3 2	3 0	6 9	6 4	24/12	28/2	93
♂	1 08	E 390			79	53	54	10 5	9 7	4 6	4 3	2 3	5 9	5 5	25/11		
11	14	R 192	0 4	97	88	74	80	10 7	6 6	4 9	3 0	2 2	5 8	3 6	20/7	23/2	67
	1 63	E 762			71	66	57	14 4	8 8	10 3	6 3	1 5	4 6	2 8	27/13		
12	[illegible]	R 224	17 4	98	80	74	76	7 2	4 3	5 5	3 3	1 3	1 7	1 0	12/5	16/0	61
♀	1 67	E 1000			56	60	44	13 7	8 2	11 5	6 9	1 2	2 2	1 3	18/10		
13	36	R 165	16 1	98	93	73	80	0 6	13 2	4 9	3 1	4 2	15 7	10 1	38/7	41/4	50
♀	1 56	E 528			83	71	59	22 0	14 1	9 2	5 9	2 4	12 8	8 2	40/12		

SVC = superior vena cava IVC = inferior vena cava s d = systolic diastolic PVR = pulmonary vascular resistance R = rest E = exercise
[a] Estimated values

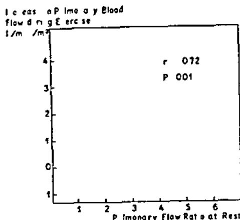


a

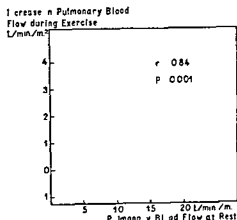


b

Fig 1 Pulmonary flow ratio at rest in relation to the percentage decrease in pulmonary flow ratio during exercise (*a*) and the decrease in shunt flow during exercise (*b*) in 13 patients with uncomplicated atrial septal defect.



a



b

Fig 2 Increase in the pulmonary blood flow durin, exercise in relation to the pulmonary flow ratio at rest (*a*) and to the pulmonary blood flow at rest (*b*) in 13 patients with uncomplicated atrial septal defect

arterial oxygen saturation was normal at rest and unchanged during exercise Therefore in the above formula (3) the O_2 saturation % of the femoral artery can be substituted for the O_2 saturation of the pulmonary veins In all the patients with the exception of no 9 the systolic pressure in the pulmonary artery was below 40 mm Hg both at rest and during exercise In the majority of patients a systolic pressure gradient was found across the pulmonary valve The systemic flow was of normal magnitude both at rest (mean 3 4 l/min/m s D 0 7) and during exercise (mean 6 7 l/min/m s D 1 1) according to the normal values from this laboratory (10) The PFR varied at rest from 6 5 to 1 3 and fell in all patients during exercise there being an obvious correlation between the PFR at rest and the percentage decrease in the PFR during exercise (Fig 1 *a*) The shunt measured in l/min fell in 11 cases whilst a doubtful increase was seen in two cases with the smallest shunts (nos 2 and 12) From Fig 1 *b* it can be seen that the decrease in the shunt measured in l/min/m was clearly dependent upon the PFR at rest.

The pulmonary flow at rest showed considerable variation from patient to patient (mean 8 9 l/min/m s D 4 6) During exercise the pulmonary flow increased in the patients with the small and medium shunts whilst the patients with the largest shunts showed an unchanged or even a slight fall in output from the right ventricle In Figs 2 *a* and *b* it can be seen that the change

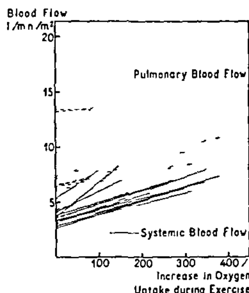


Fig 3 Changes in the pulmonary and systemic blood flow during exercise in relation to the percentage increase in oxygen uptake in 13 patients with uncomplicated atrial septal defect

in the pulmonary flow is inversely correlated both to the PFR at rest and the pulmonary flow at rest The mean value for the pulmonary flow during exercise is 10 8 l/min/m with a standard deviation of 3 3 This gives during exercise a decrease in the coefficient of variation from 52° to 31° Fig 3 shows the changes during exercise both of the systemic flow and the pulmonary flow in relation to the percentage increase in oxygen uptake The convergence of the lines indicates that the pulmonary flow during exercise increases to a lesser degree than the systemic flow and the greater the pulmonary flow is at rest the smaller is the increase in flow on exercise

DISCUSSION

In agreement with some other authors (2 8 9 12) we found in patients with uncomplicated ASD a normal systemic blood flow at rest and a normal increase during exercise though contrary to the findings of Davies and Gazetopoulos (5) There are only a few studies available on the changes occurring during exercise in the shunt of patients with ASD and normal pressure in the pulmonary artery Swan et al (11) found in two cases a fall in the PFR during exercise whilst the shunt fell in the one patient and increased in the other Scebat et al (8) found a constant decrease in the PFR whilst the shunt flow increased in five cases and fell in twenty-six whereas the pulmonary flow underwent less well defined changes Davies and Gazetopoulos measured the shunt during exercise in five patients with ASD and a normal pressure in the pulmonary artery and found no consistent changes in the shunt the PFR or the pulmonary flow

Our observations agree with the studies mentioned in the introduction in which it was found that the working capacity of the patients was independent of the PFR at rest. In the shunt both a relative and an absolute decrease related to the PFR at rest is seen during exercise despite the inaccuracy of the estimation of the pulmonary flow based upon measurements of the high oxygen saturation in the pulmonary artery and despite the difficulties in obtaining a representative sample for estimating the oxygen saturation in mixed venous blood from the venae cavae As a result of this relation between the decrease and the PFR the pulmonary flow during exercise increases to a lesser degree the greater the shunt. It even appears that it may decrease with very large shunts possibly owing to a decrease in the stroke volume of the right ventricle during the tachycardia as suggested by Jonsson et al (7) Whether changes in the shunt depend upon the intensity of the exercise a reasonable supposition has not been shown in this study as each patient was subjected to only one period of exercise and the cause of changes in the shunt during exercise in patients with uncomplicated ASD is still not clear

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where $\dot{Q}$ stands for the flow and PA the pulmonary artery Since the flow in the venae cavae is equal to the flow in the PA

$$Q_{SVC} + Q_{IVC} = Q_{PA} \qquad (2)$$

When Q_{PA} is eliminated the following is obtained

$$\frac{Q_{SVC}}{Q_{IVC}} = \frac{O_2 \text{ vol} \quad \text{in PA} - O_2 \text{ vol} \quad \text{in IVC}}{O_2 \text{ vol} \quad \text{in SVC} - O_2 \text{ vol} \quad \text{in PA}} \text{ or}$$

$$\frac{Q_{SVC}}{Q_{IVC}} = \frac{O_2 \quad \text{in PA} - O_2 \quad \text{in IVC}}{O_2 \quad \text{in SVC} - O_2 \quad \text{in PA}} \qquad (3)$$

inasmuch as vol can be replaced by the oxygen saturation percent (O_2) by dividing by the oxygen capacity and multiplying by 100

RESULTS

The results are shown in Table I From this it can be seen that at rest the mean blood flow was practically equal in the IVC and SVC the blood flow in the IVC being 48% of the total During exercise using the lower extremities the oxygen consumption increased from an average of 219 to 801 ml/min an increase of 265%, and the mean cardiac output increased from 5 2 to 9 5 l/min (3 4–6 3 l/min/m) At the same time the difference between the oxygen saturation in the SVC and IVC increased the decrease in saturation being only slight in the SVC but considerable in the IVC From Table I it can be seen that during exercise the mean blood flow in the IVC was 75% of the cardiac output as the blood flow in the IVC increased on an average from 2 5 to 7 2 l/min and decreased in the SVC from 2 7 to 2 4 l/min during exercise

DISCUSSION

Little has been written on the subject of this paper In dogs a flow was found in the IVC at rest of approximately 60% of the cardiac output (6) and in patients using Fick's principle Hultgren et al (4) obtained similar results Measurement on human subjects with krypton 85 carried out by Pannier et al (7) showed that the SVC flow decreased from 31% to 17% of the total blood flow during exercise with the legs

In patients with atrial septal defects the pulmonary flow ratio (pulmonary blood flow/

Table I *Changes in the oxygen saturation and blood flow in the superior and inferior venae cavae on exercise in 10 normal subjects*

Case no	Sex age	O_2 capacity (vol)	O_2 consumption (ml min)	O_2 saturation SVC	IVC	Pulm art	Fem art	Cardiac output (l/min)	Q_{SVC}/Q_{IVC}
	♀	?1 2	R 198	78	83	80	97	5 5	3 3/2 2
1	20		E 1068	62	38	46		9 9	3 3 6 6
	♀	19 3	R 251	70	78	74	98	5 5	2 8/? 8
2	14		E 779	64	47	50		8 5	1 5/7 0
	♀	18 0	R 2?8	75	80	77	100	5 6	3 4/2 2
3	19		E 946	78	46	53		11 1	2 4/8 7
	♂	18 2	R ?67	68	75	69	97	3 2	4 5/0 7
4	50		E 668	62	42	46		7 2	1 4/5 8
	♀	16 0	R 147	70	79	74	98	3 8	2 1/1 7
5	10		E 407	64	49	54		5 7	1 9/3 8
	o	17 7	R 146	64	73	68	98	2 8	1 6/1 ?
6	13		E 500	63	54	54		6 5	0 0 6 5
	♀	14 9	R ?57	76	81	78	97	8 9	5 3/3 6
7	21		E 954	63	53	56		15 4	4 6/10 8
	o	19 0	R 237	63	76	70	97	4 6	2 1/2 5
8	57		E 944	62	45	48		10 2	1.8 8 4
	♂	20 5	R 208	70	74	7?	96	4 2	2 1/2 1
9	12		E 612	63	50	56		7 5	3 5 4 0
	o	18 2	R 252	69	73	73	96	6 0	0 0 6 0
10	20		E 1135	64	44	49		13 2	3 3/9 9
Mean		18 3	R 219	70 3	77 ?	73 5	97 4	5 2	2 7/2 5
			E 801	64 5	46 8	51 2		9 5	2 4/7 ?

R = rest E = exercise SVC = superior vena cava IVC = inferior vena cava Q = blood flow

systemic blood flow) is calculated by the equation (2)

$$\frac{O_2 \text{ satur. of pulm. veins} - O_2 \text{ satur. of caval veins}}{O_2 \text{ satur. of pulm. veins} - O_2 \text{ satur. of pulm. artery}}$$

Our results show that the blood flows from the venae cavae are almost equal at rest. This justifies the use of the mean of the oxygen saturations in the SVC and IVC for the oxygen saturation of mixed venous blood when calculating the size of the shunt in patients with atrial septal defects. When the cardiac output is doubled during exercise with the legs, the IVC flow becomes three times as great as the SVC flow. Therefore during exercise the formula

$$\frac{(O_2 \text{ in IVC} \times 3) + O_2 \text{ in SVC}}{4}$$

should be used as the oxygen saturation percent of the mixed venous blood.

No regard has been taken in the calculations to the coronary sinus flow. This flow is approximately 5 % of the cardiac output and ignoring it does not greatly affect the results, particularly during work, as the blood in the coronary sinus is highly unsaturated.

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CELLULAR HYPERSENSITIVITY IN HASHIMOTO S THYROIDITIS

Specific Action of Thyroid Extract Upon the Migration of Leucocytes in Vitro

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Abstract An in vitro method based upon the specific action of thyroid extract upon the migration of leucocytes is presented The specificity of the method in detecting a state of cellular hypersensitivity is discussed The results presented indicate that patients with Hashimoto s thyroiditis develop cellular hypersensitivity directed against constituents of the thyroid gland

A vast number of studies on autoimmune phenomena in Hashimoto s thyroiditis have appeared during the past ten years Circulating antibodies against at least one thyroid constituent are invariably found in patients with this disease However the pathogenic importance is uncertain since the correlation between antibody titers and histological lesions is poor and because attempts to transfer thyroiditis to normal animals by means of sera containing high titers of thyroid antibodies have been unsuccessful (22 32)

Consequently the interest has been focused upon cellular hypersensitivity as a causative pathogenic factor in chronic thyroiditis The concept has been based upon the following observations in experimental allergic thyroiditis in animals

1 The lesion resembles the histology of a delayed hypersensitivity reaction in response to intradermal antigen challenge (31 32)

2 The inflammatory lesions correlate better with delayed type hypersensitivity reactions than with the occurrence and titers of circulating antibodies (14 18 19 23)

3 Experimental allergic thyroiditis has been transferred to healthy animals by means of immunocompetent cells (7) though others have not been able to confirm this observation (25)

In human beings few observations of cellular hypersensitivity to constituents of the thyroid gland in chronic thyroiditis have been published The massive infiltration of lymphocytes and other mononuclear cells in the thyroid seems to suggest a close relationship to cellular immunity

Skin reactions following intradermal injection of thyroid extracts into thyroiditis patients have been difficult to interpret because the presence of circulating antibodies suggests the reaction to be an Arthus phenomenon rather than a delayed type hypersensitivity reaction (2 33)

According to other workers intracutaneous testing as a parameter of cellular hypersensitivity is of little value in the presence of high titers of circulating antibodies (5 30)

To obtain a more specific registration of the cellular hypersensitivity the skin window technique has been applied (34) Following intradermal injection of thyroid extract an increased percentage of basophilic leucocytes was found in the exudate of patients with thyroiditis indicating that cellular hypersensitivity participates in the intradermal reaction but even in this system the interference of circulating antibodies cannot be totally excluded

Consequently it is more rational to use an in vitro method which—besides other advantages—is able also to exclude the influence of circulating antibodies Previous in vitro experiments using tissue cultures have revealed conflicting results Bjorklund (1) and Rose et al (24) demonstrated an aggressive effect of lymphoid cells from immunized animals against thyroid monolayer cultures but Ling et al (17) did not observe this effect using the same experimental model in human beings MEDICAL



Fig 1 Migration of leucocytes from a patient with thyroiditis *a* no antigen added *b* with antigen

The purpose of the present study was to apply another in vitro system based upon the specific action of antigen upon the migration of leucocytes This method originally described by Rich and Lewis (21) and later applied in man by Søborg and Bendixen (28) seems to be a specific parameter of cellular hypersensitivity

MATERIAL AND METHODS

The material consisted of 15 patients with Hashimoto s thyroiditis verified by thyroid biopsies and 25 control persons without evidence of thyroid disease

Leucocyte migration studies and determination of antibodies were performed at the same time in each individual

Leucocyte migration studies

The technique has been described in detail in a previous paper (28) White blood cells were obtained from the peripheral blood After thorough washing the cells were placed in capillary tubes then put into culture chambers and tissue culture medium was added

A thyroid extract was used as an antigen prepared according to the method described by Goudie et al (9) 100 μl of this extract was added to half of the cell cultures

The cell migration areas were measured after 4 hours and the average migration area of the antigen-containing cultures M was related to the average migration area of the control cultures M and expressed as follows viz. M /M = migration index Thus the numerical value of the migration index expresses whether the migration has been stimulated or inhibited by the antigen An index more than 1 00 means stimulation and less than 1 00 inhibition of the cell migration The appearance of a stimulated and an inhibited culture is shown in Figs 1 and 2 respectively

Demonstration of circulating antibodies

Microsomal antibodies were demonstrated by means of an immunofluorescence technique as described by Holborrow et al (13) Positive reactions were titered by means of a complement fixation reaction described by Roitt and Doniach (?2) using standardized extracts of toxic goiters as antigen Thyroglobulin antibody was demonstrated by thyroglobulin sensitized sheep cells obtained from Burroughs Wellcome & Co

RESULTS

Fig 3 shows the distribution of the migration indices of 15 patients with Hashimoto s thyroiditis and 25 control persons The observations from

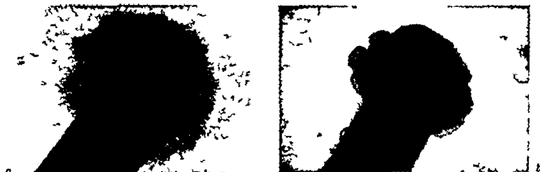


Fig 2 Migration of leucocytes from a patient with thyroiditis *a* no anti en added *b* with antigen.

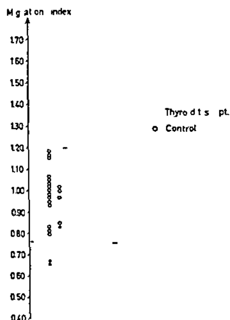


Fig 3 The distribution of migration indices in 15 patients with Hashimoto s thyroiditis and in 25 control persons

the patients with thyroiditis seem to fall in three categories one characterized by a marked inhibi tion of the migration another by a stimulation and a third showing neither inhibition nor stimu lation When the indices of the thyroiditis patients are looked upon as one group the mean value is 1 02 and the standard deviation 0 34 while the mean value of the controls is nearly the same i e 0 98 but with a standard deviation

Table I *Circulating thyroid antibodies and migration indices of 15 patients with Hashimoto s thyroiditis*

Pat. no	Cytoplasmic fluorescence	Complement fixing titer	Thyroglobulin antibody	Migration index
1	+ +	256	2 500 000	0 44
2	+ +	–	25 000	0 60
3	∸ +	32	250	0 60
4	∸	512	2,500 000	0 62
5	+	32	250	0 66
6	–	32	25 000	0 71
7	+ +	64	25 000	0 84
8	+ + +	–	250 000	0 84
9	+ +	256	2,500	0 89
10	+	1 8	> ..500 000	1 30
11	(+)	–	> ..500 000	1 33
12	–	–	? 500	1 33
13	∸	64	.50 000	1 53
14	(∸)	16	250	1 58
15	–	–	25 000	1 72

of 0 11 This difference in the standard devia tion means that the observations from the thyroid itis patients and the controls form two signifi cantly different populations ($f = 8$ 53)

Table I shows the findings of circulating anti bodies and the corresponding migration indices of the thyroiditis patients It appears that there is no correlation between migration indices and titers of either the microsomal antibody or the thyroglobulin antibody In the controls low titers either of thyroglobulin antibody (T R C) or microsomal antibodies were demonstrated in about 30 % of the sera

DISCUSSION

According to the generally accepted view the ac tion in vitro upon immunocompetent cells re sults in an inhibition of the cell migration if the cells originate from an organism in a state of cellular hypersensitivity to the same antigen This observation has been confirmed in many animal experiments using different antigens and various kinds of immunocompetent cells (3 4 6 8 12 15 20 21 26)

The above in vitro method has recently been applied in studies in man using Brucella bacteria as antigen (29) The results were in accordance with the animal experiments and showed the leucocyte migration inhibition induced by bru cella antigen to be a specific expression of cellular hypersensitivity well correlated to the intracu taneous reaction The concept however that an inhibition of the cell migration exclusively is an indication of cellular hypersensitivity is not quite correct. A few experiments indicate that in some instances a stimulation of the cell migration may be observed as well (16 27) This phenomenon has also been noticed in experiments with bru cella hypersensitivity (unpublished observations) If high concentrations of the antigen were used (i e 50 mill bacteria per ml) the result was al ways an inhibition of the migration If the antigen concentration however was gradually lowered the result was at first a disappearance of the in hibition but at a sufficiently low antigen con centration a significant stimulation of the migra tion was observed At a certain relatively low *antigen concentration (i e 1 mill bacteria per* ml) a wide distribution of indices was observed indicating that the migration had in some cases

been stimulated in others inhibited and finally in some cases left uninfluenced by the antigen The migration indices of brucella negative controls were all grouped around 1 00 with minor fluctuations A further analysis of these migration indices showed that the inhibition at the low antigen concentration corresponded to a high degree of cellular hypersensitivity and the stimulation corresponded to a low degree of sensitivity The observations within the normal range corresponded with an intermediary degree of hypersensitivity

The present results show that the migration indices of the thyroiditis patients were spread within a wide area indicating a stimulation as well as an inhibition of the leucocytes upon contact with the thyroid antigen Three of the observations are within the normal range The distribution of the indices is very similar to the distribution observed in the experiments with brucella hypersensitivity at the low antigen concentration This similarity suggests that the effect of the thyroid antigen upon the leucocyte migration expresses a state of cellular hypersensitivity with varying degrees of sensitivity As to the three observations within the normal range it is impossible to say whether these indicate a medium degree of sensitivity or no sensitivity at all

In analogy with the brucella experiments the data presented here indicate that the concentration of the thyroid antigen is too low to elicit a migration inhibition in all cases of cellular hypersensitivity to thyroid antigen It would seem logical to increase the concentration of the thyroid antigen until an inhibition of the migration took place in all cases of cellular hypersensitivity In this way a more precise quantitation of the sensitivity should probably be obtained Indeed this approach was tried but unfortunately the higher antigen concentration affected the migration of the control cultures non specifically To solve this problem it will be necessary to obtain a more purified antigen solution than the rather complex extract which has been used in these experiments A separation and purification of the various substances of the thyroid extract would also permit identification of the antigen(s) responsible for the recorded cellular hypersensitivity

As previously mentioned the experimental conditions are not ideal because of the rather low antigen concentration which had to be used Nevertheless it seems reasonable to conclude that the data presented indicate a state of cellular hypersensitivity directed against substances of the thyroid gland in Hashimoto s thyroiditis The results do not allow the conclusion that cellular hypersensitivity is present in all cases since three observations fell within the normal range It seems reasonable to assume however that future experiments using a more purified antigen will reveal that cellular hypersensitivity to the thyroid gland is a constantly occurring phenomenon

The presence of circulating thyroid antibodies in a rather high percentage in the controls is in good agreement with the findings in larger control materials (10 11) In contrast no cellular hypersensitivity was demonstrated in the 25 controls On the other hand it was not possible to demonstrate cellular hypersensitivity in all thyroiditis patients owing either to the impurity of the antigen or to low sensitivity of the method

The lacking correlation between the migration indices and the antibody titers shows that the method applied is able to distinguish between humoral and cellular hypersensitivity which is in good agreement with previous experiments in brucella hypersensitivity (29) Furthermore the discrepancy is demonstrated between these two types of hypersensitivity which shows that a registration of both kinds of immunological reactivity is necessary in the study of Hashimoto s thyroiditis

Whether the cellular hypersensitivity is more closely related to the pathogenesis of Hashimoto s thyroiditis than the circulating antibodies is dubious and the data presented do not allow any conclusions in this respect

Under all circumstances it must be kept in mind that the presence of immunological phenomena does not tell much about their pathogenetic role and the possibility still exists that they are secondary to a primary unknown damage to the thyroid gland

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FAMILIAL SERUM CHOLESTEROL ESTER DEFICIENCY

Clinical Study of a Patient with a New Syndrome

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Abstract Three adult sisters with only traces of esterified cholesterol in serum are reported All had proteinuria and anemia Elevated total cholesterol triglyceride and phospholipid concentrations were present in serum of the two elder sisters All three had decreased amounts of serum lysolecithin None of them had symptoms of liver disease

The oldest sister 33 years was subjected to a thorough study She had marked lipoid deposits in corneae normal tonsils and normal sized liver and spleen She had a marked normochromic anemia due to slight hemolysis and reduced ability to compensatory increase of red cell production. A constant proteinuria of moderate degree was present Serum creatinine and electrolytes were normal Liver function tests revealed no abnormalities Serum uric acid was elevated

No α lipoproteins in serum could be detected by lipoprotein and immuno-electrophoresis Lipoprotein electrophoresis revealed a broad band in the β position with marked trailing into the chylomicron area

Foam cells were found both in the bone marrow and in the glomerular tuft of the kidney

The constellation of symptoms and findings is believed to represent a familial syndrome not previously described

Normally 60–80% of the total cholesterol in serum is esterified with long-chain fatty acids (9) Selective reduction in serum cholesterol ester has been reported in some patients with acute febrile infections (17 18) and in some types of familial hyperlipoproteinemias (7) Marked decrease of the ratio esterified/free cholesterol has however till now been found only in patients with severe hepatic parenchymal disease (2 3 14 19) We have recently studied three adult sisters all of whom had only traces of esterified cholesterol in serum All three had proteinuria and anemia but only the oldest one was clinically ill She was subjected to a clinical study which in addition to serum lipid and lipoprotein abnormalities revealed lipid deposits in corneae foamy cells in the bone marrow and kidney glomeruli proteinuria normochromic anemia hyperuricemia and possibly increased glycogen content of the liver cells The details of this study are reported here

CASE REPORT

Our patient (A. R) is a female born in 1933 from a district in Western Norway Both parents are alive and unrelated Her father is 7? year-old and in good health He probably had a minor coronary infarction in 1964 His first wife died young of tuberculosis They had four healthy children—all daughters

The patient is the oldest of five sisters in the father s second marriage She knew of no inherited disease in her parents families Her mother is healthy

Two sisters of our patient are known to have proteinuria I S (born 1935) was hospitalized for this reason in 1955 She has three healthy children a 9 year old healthy son and two 7 year-old twin boys

The proteinuria of the other sister M R (born 1947) was discovered in 1964 No particular disease is known among the other sisters or half-sisters

The proteinuria in our patient was discovered in May 1952 and led to hospitalisation The urine contained approximately 0.5 mg/ml protein Proteinuria was also present in the fall of 1953 during a short stay in hospital on a suspicion of poliomyelitis acuta In 1955 during a normal pregnancy protein was present in urine at all controls Delivery was normal but the full term daughter died the next day In 1959 she had a second normal pregnancy with constant proteinuria A normal daughter was born by Caesarean section Blood pressure had on all these occasions been normal Otherwise she has been healthy and no control of urine or kidney function was made until April 1966 when she felt an increasing general asthenia and noticed ankle edemas A general practitioner again found proteinuria and a marked anemia (Hb 7 6 g/100 ml) She was therefore admitted to our hospital in June 1966 and in January 1967

On the last admission she was 33 years old 178 cm tall and 67 kg in weight. She was pale and had moderate

Table I *A R Laboratory values on admission January 1967*

ESR (mm/h)	61	Potassium (mEq/l)	4 5
Hemoglobin (g/100 ml)	8 7	Chlorides (mEq/l)	102
Red blood cells (mill/μl)	2 92	Calcium (mEq/l)	4 2
White blood cells (μl)	4500	Phosphorus (mg/100 ml)	4 7
Platelets (/μl)	113 000	Alkaline phosphatase (int units)	.7
Reticulocytes (/1000 red cells)	1–16	Acid phosphatase (int units)	14 0
Serum iron (μg/100 ml)	55	pH	7 4?
Transferrin (μg/100 ml)	140	Standard bicarbonate (mEq/l)	23
Serum values		pCO_2 (mm Hg)	36
Urea (mg/100 ml)	58	SGOT (Karmen units)	27
Uric acid (mg/100 ml)	8 6 & 10 0	SGPT (Karmen units)	18
Creatinine (mg/100 ml)	1 1	Thrombotest (of normal)	>100
Sodium (mEq/l)	135	PBI (μg/100 ml)	6 2

ankle edemas Marked lipoid arcus were present in both corneae but no xanthomatous deposit was found in skin or tendons Blood pressure was normal 145/95–1.0/80 mm Hg Physical examination revealed no abnormalities in the lungs heart or abdomen The tonsils were not enlarged and had a normal appearance Liver spleen and kidneys were not palpable The reflexes were normal She had normal joints and no uric acid tophi

1 *Laboratory values* on admission in January 1967 are given in Table I Normal values were found for electrolytes calcium phosphorus alkaline phosphatase creatinine liver function tests pH pCO standard bicarbonate and protein bound iodine (PBI) Serum urea and acid phosphatases were slightly elevated and uric acid increased

? *Serum l pids*

results of examination of the major serum lipid are given in Table II *a* Our patient (A R) and her sister (I S) had elevated values of total cholesterol (1) phospholipids (4) and triglycerides (11) whereas the youngest of the three (M R) had normal values for all these lipid fractions All three had almost identical values of total and free cholesterol when an analytical method (16) employing digitonin precipitation was used Thin layer chromatography revealed traces of esterified cholesterol (15) Determination of individual serum phospholipid fractions (8) revealed abnormally low lysolecithin values in all three sisters (Table II *b*) In serum from A R lysolecithin P represented only 1 3 % of a total lipid phosphorus of 159 5 μg P/ml The serum lipoprotein electrophoretic patterns (1?) of the three sisters revealed that no α or pre β bands were present in any of them Sera from A R and I S both showed marked bands in the β position with abundant trailing into the chylomicron area (15)

3 *Other biochemical findings*

Urinalysis showed spec grav 1006–1016 A constant proteinuria of very moderate degree—from traces up to 1

Table II*a* *Serum lipid fractions*

Pat	Age (y)	Cholesterol (mg/100 ml) Total	Free	Triglycerides (mg/100 ml)	Phospholipids (mg 100 ml)	Free fatty acids (mEq/l)
A R	33	302	292	312	400	0 70
I S	31	580	550	573	810	0 46
M R	20	143	136	129	165	0 41

Table II*b* *Serum phospholipid fractions*

	Lysolecithin (μgP/ml)	( )	Sphingomyelin (μgP/ml)	( )	Lecithin (μgP/ml)	( )	Cephalin (μgP/ml)	( )	Sum of fractions (μgP ml)
A R	2 1	1 3	15 4	9 7	135 7	85 1	6 3	3 9	159 5
I S	5 9	1 8	36 6	11 3	269 5	83 3	11 8	3 6	3.3 8
M R	1 7	2 0	9 7	11 6	69 3	82.8	3 0	3 6	83 7
Normals									
Mean	3 8	5 5	1.0	17 3	50 2	73 1	2 9	4 1	68 9
Range	2.6–7 2	3 7–9 3	8 .–16 6	12 6–21 8	44 0–53 6	68 6–77 5	2.1–3 5	2 9–5 6	60 3 77.2

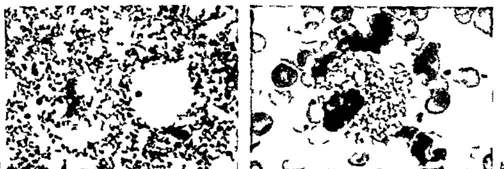


Fig 1 Bone marrow aspirate from A R. demonstrating ( ) normal cellularity and (*b*) one of the typical foam cells May Grunewald Giemsa staining

mg/ml was present Electrophoresis of urine protein showed that 70% was albumin and 30 globulin Other wise the urine was chemically normal Granulated casts were found occasionally Lipid extraction of the urine and subsequent thin layer chromatography did not de monstrate any abnormal lipid material compared to nor mal urine Six Loewenstein cultures for tb were nega tive

Serum p oteins were electrophoretı ally determined as albumin 5 α globulin 0 3 α_2-globulin 0 8 β globulin 0.5 and γ-globulin 1.2 g/100 ml respectively

AST 1 0 AStaT 1 WR negative LE-cells were not demonstrable Blood group 0 Rh positive

Vitamin A test revealed normal fat absorption —5737 I units vitamin A/100 ml serum four hours after an oral load of 350 000 I units of vitamin A palmitate

Bromsulphalein tests was not abnormal—9 retention after 45 min but serum was turbid

Galactose test was normal as the half time was 9 min after intravenous injection of 0 35 g Galactose Kabi per kg body weight (Normal value 12±2 min Pathological value = 17 min)

Glucose tolerance curve was normal after oral load of 67 g glucose with increase of blood glucose from 65 mg/100 ml to 128 mg/100 ml after 30 min and falling to 76 mg/100 ml after 60 min

Glucagon test showed normal increase of blood glu cose after i v injection of 1 mg Glucagon Novo—from 80 to 1 4 mg/100 ml in 15 min

4 *Hematological examinat ons*

( ) Table I demonstrates that the patient had a marked normochromic anemia moderate sideropenia very low transferrin and a moderate thrombopenia Differential white count showed 2% basophils 5% eosinophils 56% segmentated neutrophils and 37% lymphocytes Osmotic fragility was normal both in fresh blood (maximal re sistance 0 3 % NaCl and minimal resistance 0 45% NaCl) a d after incubation for 24 hours at 37 C (max res 0 3% NaCl min res 0 75 NaCl) Coombs test was negative Haptoglobin was present but d fficult to quan titate because of the turbidity of the serum

The concentration of vitamin B_{12} in serum was 76 picogram/ml (normal value) Schilling s test showed nor mal absorption of vitamin B_{12} as 4 of given dose wa excreted in urine in the first 24 hours Bleeding time was 9–10 / min Coagulation studies revealed no abnorma lities in the intrinsic or extrinsic coagulation systems PP 100% Quicktime 16 0–15 0 sec Cephalin time 6 6–63 2–61 sec Platelet adhesiveness in citrated whole blood was 24 (low normal value)

(*b*) *The bone marrow* was studied both after aspiration and in a cylinder biopsy The cellularity of the marrow was normal (Fig 1 *a*) Erythropoiesis represented 25 of the nucleated cells and was normoblasti There was a slight increase in the number and immaturity of the erythroid cells Myelopoiesis was normal apart from a slight eosinophilia Lymphocytes and plasma cells were within normal limits and also the number of reticulum cells was normal Megacaryocytes were present in normal amounts Large foamy cells were seen scattered around in the marrow (Fig 1 *b*)

(*c*) *Radio sotope studies* of the erythrocyte life span and the iron metabolism were performed Erythrocytes from 25 ml of the patient s blood were labelled with 0 μCi Cr O — and injected intravenously suspended in isotonic saline At the same time approx 10 μCi ^{59}Fe-citrate was dissolved in the patient s plasma and injected i v

(i) *Blood volume determinations*

Total blood volume	5670 ml or 118%	of normal
Erythrocyte volume	1401 ml or 86	of normal
Plasma volume	4269 ml or 136%	of normal

( ) *Plasma iron tu nover*

Half time of plasma iron was found to be 60 m n (normal 80–120 min) corresponding to an hourly ex change of 69 of the total plasma iron (normal 35–50) Plasma iron transport rate was determined to be 39 mg/24 hours (normal approx 30) or 21 μg/kg body weight/h (normal approx 18)

By surface scanning (Fig 2 *a*) of ^{59}Fe over liver spleen heart and os sacrum for 22 days after the injection a normal increase in activity over os sacrum was found after 4 hours whereafter the activity decreased rapidly during the next 21 days The activity over the heart, and

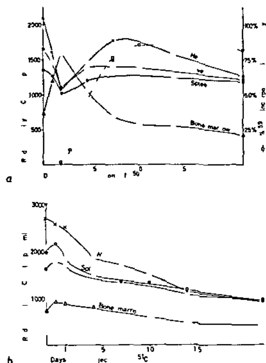


Fig 7 Surface scanning of ^{59}Fe (*a*) and Cr labelled erythrocytes (*b*) over liver spleen heart and os sacrum of A R after injection of 10 μ Ci ^{59}Fe-citrate and erythrocytes labelled with 50 μ Ci Cr O

a lesser degree over liver and spleen increased during first seven days after the injection and thereafter de eased slowly and linearly All these surface registrations therefore showed normal results

Calculations of ^{59}*Fe incorporation in erythrocytes showed* a normal gradual increase durin the first ten days when maximal incorporation was 85 of injected ^{59}Fe which is normal

(iii) *E ythrocyte life span*

Half time for ^{51}Cr labelled erythrocytes was found to be 16 days Surface scanning of Cr over heart liver spleen and os sacrum demonstrated a gradual fall of activity over the 22-day period with normal distribution between these organs (Fi 7 b)

These investigations therefore demonstrated a slightly re duced erythrocyte volume slightly increased blood vol ume and a more marked increase in plasma volume Plasma iron transport rate was normal with reduced half time for plasma iron due to sideropenia Erythropoiesis was normal The life span of erythrocytes was moderately reduced indicating some degree of hemolysis There was no indication that the spleen was particularly active in erythrocyte destruction.

(*d*) *The hematological evaluation* including the bio- chemical data the isotope studies and the morphological characteristics have thus shown a marked normochromic anemia that has two components—a slight hemolysis and a reduced ability to compensatory increase of red cell production

5 *Supplementary examinations*

Analytical ultracentrifugation of serum in Michaelis buf fer with protein concentration of 1 5 g/100 ml and added 2% NaCl gave the following results

Component 1 (mainly albumin)
sedim coeff 3 6 S conc 4 09 g/100 ml
Component 2 (*mainly* γ *globulin*)
s dim coeff 5 6 S conc 0 9 g/100 ml
Component 3 (macro globulin)
sedim coeff 14 5 S conc 0 51 g/100 ml

No abnormal component was demonstrated The con centration of the normally occurring macroglobulin (14.5 S) was increased above the upper normal limit of 0 36 g/100 ml whereas the absolute concentration of albumin was below the lower normal limit of 5 g/100 ml

Serum immunoelectrophoretic studies showed a slight in crease of γ-M globulin but did not demonstrate any α lipoproteins (for details see (15))

Electrocardiogram (12 leads) was normal *Electromyo- grams* from both m quadriceps and both m tibialis anti cus and right m biceps were normal The peripheral *nerve conductivity* was also normal

X ray revealed a heart of normal size (355 ml/m body surface) and shape Intravenous pyelogram showed en larged kidneys (approx 17-7 5 cm) on both sides with somewhat dilated calyces

6 *Histological examinations*

A needle renal biopsy showed (July 1966) clear swollen *cells of the glomerular tuft It could not be definitely* established whether these cells were epithelial or endo- thelial They did not contain glycogen or amyloid but resembled the foamy cells seen in the bone marrow

A needle liver biopsy suggested increased amounts of glycogen within the liver cells by ordinary stains a find ing which was confirmed by positive Best s carmine stain with negative saliva control (B Öystese Institute of General and Experimental Pathology University Hospital Oslo)

DISCUSSION

Our patient demonstrated a constellation of clinical findings biochemical and pathologic anatomical abnormalities that to our knowledge has not previously been described—α and pre β lipoprotein deficiency combined with increased concentrations of total cholesterol triglycerides and phospholipids and an almost complete lack of esterified cholesterol in serum proteinuria normochrome anemia lipid deposits in corneae

foamy cells in the bone marrow and kidney glomeruli and possibly increased amount of glycogen in liver cells

We first had to consider whether these findings could be parts of previously known syndromes —in particular the nephrotic syndrome familial hyperlipoproteinemias or dyslipoproteinemias glycogen deposition diseases glycolipid lipidosis or hepatic parenchymal disease

Our patient had proteinuria of long duration It had once been diagnosed as a sub-chronic nephritis A nephrotic syndrome was therefore considered on admission as an explanation of her proteinuria normochromic anemia hypertriglyceridemia hypercholesterolemia and hyperphospholipidemia The nephrotic syndrome may according to Fredrickson et al s classification (7) present a variety of lipoprotein patterns—types II III IV and V The clinical picture however did not fulfil the criteria for a nephrotic syndrome since the proteinuria was constantly of a very moderate degree and never exceeded 1‰ her serum albumin was reduced but never below 2 5 g/100 ml edemas were only occasionally present and granulated casts were only found occasionally in the urine The histological examination of the kidney biopsy did not reveal any changes characteristic of chronic glomerulonephritis or genuine lipoid nephrosis We therefore believe that the serum lipid changes in our patient are not secondary to a nephrotic syndrome

An attempt was made to classify the elevated cholesterol and marked increase of triglyceride concentration in serum into the known types of primary hyperlipoproteinemias We could however demonstrate neither α nor pre β lipoproteins by lipoprotein electrophoresis in our patient She could therefore not be classified into any of the five main groups of Fredrickson et al (7)

A deficiency of α lipoprotein is present in Tangier s disease and these patients may have increased amounts of serum triglycerides (5) These patients however have low serum cholesterol and abnormal percentage of esterified cholesterol has not been reported Furthermore the pathognomonic feature of the large sized and orange colored tonsils (6) was not present in our patient

Hyperlipoproteinemia is found in patients with glycogen storage disease (6)—the lipid abnormality most often found being an increase of pre β lipoproteins The finding of possibly increased amounts of glycogen in our patient s liver cells combined with elevation of serum cholesterol and triglycerides raised the question of a glycogen storage disease However our patient s liver was of normal size fasting blood sugars were normal glucose tolerance was normal glucagon injection gave normal blood sugar response galactose test was normal and no glycogen deposition was found in the kidneys Heart muscles skeleton muscles and peripheral nerves were functionally normal A glycogen storage disease was therefore not the primary illness of our patient

Angiokeratoma corporis diffusum (Fabry s disease) is a very rare entity and is now accepted as a glycolipid lipidosis with intracellular accumulation of trihexose and dihexose ceramide in the kidneys and also in other organs and in the bone marrow (10 13) The kidney biopsy in our patient showed possible similarities with those from patients with Fabry s disease None of these patients however have been described with hyperlipemia nor with increased glycogen in the liver

A remarkable finding in our patient and two of her sisters was the almost complete lack of esterified cholesterol This has hitherto only been seen in very severe liver diseases All the liver function tests performed on our patient were normal We may therefore exclude parenchymal liver disease as the cause of this cholesterol esterification deficiency The two sisters with the same abnormality have never been clinically ill and they have never suffered from any liver disease It therefore seems justified to believe that this cholesterol ester deficiency is familial We further believe that the described constellation of symptoms and findings in our patient represents a genuine familial syndrome not previously described

More detailed studies of the lipoprotein abnormalities and the plasma cholesterol esterifying system of the patient and her family are in progress The results of these studies will be published separately (15)

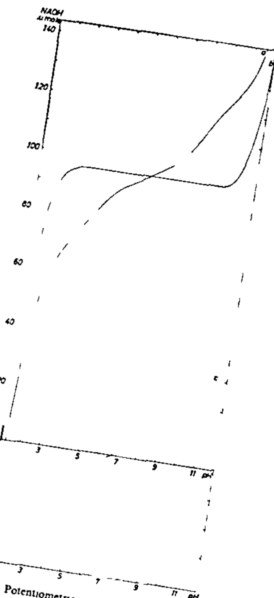


Fig 1 Potentiometric titration curves *Abscissa* pH *ordinate* sodium hydroxide in µmole (*a*) 750 µl gastric juice to which had been added 100 µl 1 N hydrochloric acid (*b*) 750 µl sodium chloride solution to which had been added 100 µl 1 N hydrochloric acid (*c*) Buffer substances in 750 µl gastric juice

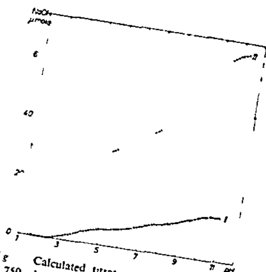


Fig Calculated titration curves for buffer substances in 750 µl gastric juice before (I) and after (II) administration of glycopyrrolate to a 40-year-old woman with gastric ulcer

which 100 µl 1 N hydrochloric acid had been added (Sodium and potassium in the original gastric juice had previously been measured by flame photometry)

Subtraction of the blank titration curve (b) from the titration curve representing the gastric juice (a) gave a difference curve (Fig 1 *c*) which represents the titration of the buffer substances of the gastric juice specimen The subtraction was carried out at pH intervals of 0 1–5

When disregarding whether the acidified gastric juice contains a larger or smaller number of hydrogen ions than the blank the starting point of titrating buffer substances—as done in the following—may be stated as zero point

EXPERIMENTAL

From two patients with peptic ulcer gastric juice was collected before and two hours after intramuscular injection of 0 5 ml glycopyrrolate (1 mg ml) These samples were treated and titrated as described above

RESULTS

The titration curves calculated for buffer substances in the gastric juice of two patients with peptic ulcer before and after administration of glycopyrrolate are shown in Figs 2 and 3

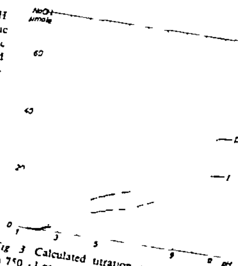


Fig 3 Calculated titration curves for buffer substances in 750 µl gastric juice before (I) and after (II) administration of glycopyrrolate to a 43 year-old man with duodenal ulcer

The curves in Fig 2 are from a 40-year-old woman with gastric ulcer The pH in samples collected before (I) and after (II) administrations of glycopyrrolate was found to be 2 75 and 7 05 It is apparent from the curves that the titration of the buffer substances started at 2 5 and 1 6 pH respectively

The curves shown in Fig 3 are from a 43 year old man with duodenal ulcer The pH in the samples collected before (I) and after (II) administration of glycopyrrolate was 1 55 and 4 05 respectively The titration of the buffer substances started at pH 2 2 for both samples

Administration of glycopyrrolate increased the number of hydrogen ions that could be bound in the same volume of gastric juice in the two patients—by 350 o in the patient with gastric ulcer and by 50° in the patient with duodenal ulcer

All the calculated titration curves for the buffer substances of the gastric juices showed identical buffer areas with a maximum buffer capacity around pH 3 5 7 5 and 9 5 and the titration of the buffer substances appeared to have been completed at pH 10 5–11

DISCUSSION

After administration of glycopyrrolate there was an increased consumption of sodium hydroxide for titration of buffers (Figs 2 and 3) indicating an increase in the concentration of buffer substances of 350 o and 50 respectively

The investigation comprised only non volatile buffer substances carbon dioxide bicarbonate being removed from the gastric juices by the acidification and bubbling with nitrogen prior to the titration Therefore the studied buffer substances must in all essentials consist of mucoid substances including their possible degradation products since other inorganic substances such as phosphates occur in very small amounts in gastric juice

Care was taken that the difference in the alkali concentration in the gastric juice and the blank sample should not exceed 15 mEq/l at the institution of the titrations (4) On the other hand no correction was made for the higher alkali concentration which had to be present at a later stage of the titrations in gastric juices thanks to their greater consumption of sodium hydroxide The reason for this omission is that an unknown large share of extra sodium added at the titration of the gastric juice will be bound to the buffer substances of the juice and thus be osmotically inactive (3) At the low pH in the beginning of the titration potassium and sodium were present as free ions

The areas in the calculated titration curves with maximum buffer capacity around pH 3 5 7 5 and 9 5 must be due to the accumulation of protolytic groups in the buffer substances with pK values at the named pH s The steepness of the curve in the individual buffer areas is a measure of the concentration of protolytic groups in the individual areas Curves having an identical mutual inclination in the three areas indicate that the titrated buffer substances have been of the same qualitative composition

In spite of widely different pH s in the secretions that were aspirated partly without and partly during the influence of glycopyrrolate the buffer substances of the gastric juices appear to be of the same composition (cf titration curves in Figs 2_{II} and 3_{I} and $_{II}$) Admixture of saliva cannot be ruled out in the specimen whose titration curve of buffer substance is illustrated in Fig 2_{I} Thus unlike the findings of Plummer et al (7) the present study does not indicate that an anticholinergic substance causes a qualitative change in the buffer substances of the gastric juice

The full extent of the titration curves for the buffer substances of the gastric juices is required as a link in identifying the buffer substances The physiological role of the hydrogen ion binding of the buffer substances on the other hand manifests itself at a pH below 8

Division of titration curves for acidified gastric juice into two parts 1 titration curves for free hydrochloric acid and 2 titration curves for buffer substances has proved useful for assessing the influence of glycopyrrolate upon the concentration of buffer substance At the same time it gives the key to an increased understanding of titration results on gastric juice on the whole

ACKNOWLEDGEMENT

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SPLENECTOMY IN HEMATOLOGIC DISEASES

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Abst act The effect of splenectomy in a variety of hematological disorders has been evaluated by a follow up study of 179 patients 3–15 years after surgery A lasting remission was obtained in about 75% of patients with id opathic thrombocytopenic purpura All patients with hered tary spherocytosis had a complete remission whereas hered tary non-spherocytic hemolytic anemia was not influenced by splenectomy More than 50% of pa tients w th acquired hemolytic anemia showed no symptoms of hemolytic disease when examined ten years after surgery Twenty five of the patients with pancytopenia were in good condition ten years after splenectomy Normal or increased bone marrow cellularity in this condition strengthens the indication for surgery The proliferative diseases including chronic lymphatic leu kemia, lymphosarcoma and myelofibrosis may represent ind cations for surgery whereas chronic myelogenous leukemia seemed to be a contraindication for splenectomy The hematological disturbances associated with congestive splenomegaly usually can be corrected by splenectomy

Splenectomy is an accepted therapy in certain hematological disorders Yet our knowledge of the physiology and the pathophysiology of the spleen remains poor Removal of the spleen be cause of a traumatic lesion in otherwise healthy individuals seems to be without adverse effects (20) Indications for splenectomy in non traumatic diseases have been based on clinical experience but are still poorly defined Further observations on the fate of such patients may improve the criteria for splenectomy and also provide in formation on the role of the spleen in various diseases

The present report concerns clinical experience of splenectomy at the Surgical Department B Rikshospitalet during the period 1952–1964 The follow up was carried out in 1967 giving an ob servation time from 3 to 15 years The pre operative study and the reassessment of most pa tients took place at the Medical Department A Rikshospitalet

MATERIAL

The series comprises 179 patients who had splenectomy performed for the hematological disorders listed in Table I The main indications were thrombocytopenia hemolytic anemia and pancytopenia These syndromes were caused by non malignant as well as malignant proliferative hematologic disorders The operative procedure has recently been described (18) All patients with a platelet count exceeding 400 000/mm post-operatively were routinely given anticoagulant treatment.

One hundred and two or 57 of the patients were alive at the follow up Of these 70 were examined at our hosp tal Sixty three or 35% of the patients were dead Most of these patients had d ed at home and autopsy had not been performed For some of them in formation was obtained from other hospitals Fourteen patients or 8 could not be traced at the time of follow up

RESULTS AND COMMENTS

Results of the hematological control as com pared with values found before splenectomy are summarized in Tables III–X.

Thrombocytopenia

Idiopathic thrombocytopenic purpura

The mean age and the sex distribution are given in Table II Nine of the patients were less than 15 years of age at operation In none of the patients was the occurrence of symptoms associated with acute infections transfusions or the use of drugs The average duration of symptoms before operation was 12 months Thirty four of the patients were treated with steroids before operation and the daily dosage of prednisone varied from 1/ to 2 mg/kg Nearly half of the patients did not respond to this treatment as shown in Table III *b* In most patients who did respond the dosage was much too high to be used over a longer period of time

Table I *Splenectomy at Surgical Dept B Rikshospitalet 1952–1964 Status 1967*

Diagnosis	Total no	Alive	Deaths	Fate unknown
Thrombocytopenia				
Idiopathic thrombocytopenic purpura	43	37	5	1
Cong macrothrombocytic thrombocytopenia	3	3	—	—
Hemolytic anemia				
Hereditary spherocytosis	33	25	1	7
Non spherocytic hemolytic anemia	2	2	—	—
Acquired hemolytic anemia	18	10	7	1
Pancytopenia	35	9	23	3
Proliferative disorders				
Chronic lymphatic leukemia	9	1	8	—
Chronic myelogenous leukemia	5	—	5	—
Reticulosis	9	3	5	1
Myelofibrosis	3		3	
Felty s syndrome	2	1	1	
Congestive splenomegaly	17	11	5	1
Total	179	102	63	14

In this group of thrombocytopenia the spleen was seldom enlarged Two patients only had a definite splenomegaly (Table II) None of these patients had serious postoperative complications All patients were given platelet rich plasma or platelet concentrates from two or four donors immediately before and during the operation

At the follow up (Tables I and III *a*) 37 out of the 43 patients were examined Thirty two had obtained complete remission and no bleeding episodes had occurred after the operation The other five were in good condition without symptoms of bleeding but the platelet count ranged from 16 000 to 100 000/mm³ and three of them were on continuous steroid treatment Five patients had died two of these because of severe infections septicemia and meningitis one and two years after splenectomy both had normal platelet counts Two patients died of cerebral vascular insults five and seven years after splenectomy In one of these thrombocytopenia was present Finally one patient died one month after surgery with severe thrombocytopenia and a possible malignant infiltration in the right lung

To summarize 75% of the patients showed a complete remission at an average observation

Table II *Age and sex distribution preoperative duration of symptoms and weight of spleen at operation*
Mean values and range

Diagnosis	Mean age at operation	Sex ♂	Sex ♀	Duration of symptoms (months)	Weight of spleen (g)
Thrombocytopenia					
Idiopathic thrombocytopenic purpura	3[illegible] (2–25)	15	28	1[illegible] (4–120)	170 (50–640)
Cong macrothrombocytic thrombocytopenia	13 (6–17)	2	1	—	185 (45–275)
Hemolytic anemia					
Hereditary spherocytosis	22 (1 60)	22	11	—	553 (75 1500)
Non spherocytic hemolytic anemia	10 (4–15)	2	0	—	285 (2[illegible]0–385)
Acquired hemolytic anemia	50 (14–70)	7	11	[illegible]4 (4 144)	540 (160–1400)
Pancytopenia	37 (4–75)	14	21	29 (5–144)	268 (3 970)
Proliferative disorders					
Cr lymphatic leukemia	58 (3[illegible]–65)	8	1	35 (–132)	910 (5 0–[illegible]190)
Cr myelogenous leukemia	54 (32–69)	3	2	25 (4–60)	850 (1 0–[illegible]080)
Reticulosis	58 (41–73)	4	5	30 (6–60)	1 85 (185–3000)
Myelofibrosis	66 (63–69)	2	1	48 (4–72)	1530 (1[illegible]50– 100)
Felty s syndrome	50 (46–54)	1	1	10 (8 12)	1050 (970–11 0)
Congestive splenomegaly	26 (5–58)	6	11	6[illegible] (6–31)	735 (125–1150)

Table IIIa *Idiopathic thrombocytopenic purpura*

Hematologic condition before and an average of 7 3 years after splenectomy in the surviving 37 patients

	No	Erythrocytes (×10⁶/mm³)	WBC (/mm)	Platelets (/mm³)	Hb (g 100 ml)
Before operation	37	4 5 (2 8–6 3)	7300 (2200–19 100)	10 700 (800–100 000)	13 (9 6–16 1)
7 3 (3–15) years after operation	37	4 7 (3 5–5 8)	8300 (?700–17 800)	249 000[a] (16 000–600 000)	13 9 (11 8–16 0)

Fi e patients had below 100 000 platelets mm

Table IIIb *Idiopathic thrombocytopenic purpura*

Effect of prednisone on platelet count before operation The dose of prednisone was ½–2 mg/kg/day and the treatment lasted for an a erage of 3 months

Total no of patients	Effect of prednisone: None	Transient	Good	Not given prednisone
43	19	9	6	9

time after splenectomy of seven years This is in accordance with the findings of Carpenter et al (5) but somewhat higher than reported by Baldini (2) The observation time is so long that a lasting effect might be expected It is known that even in chronic idiopathic thrombocytopenic purpura spontaneous remissions do occur Recurrences however are frequent, and fatal relapses have been reported (27)

The use of corticosteroids in the treatment of chronic idiopathic thrombocytopenia has been discussed by Baldini (2) The present study suggests that long term treatment should be abandoned because the beneficial effect in most cases is doubtful and the side effects are often serious Even short term treatment before splenectomy in order to increase the platelet number at operation seems to be of doubtful value although a decreased bleeding tendency has been observed after steroids even without increase in the platelet number If there is no remission following splenectomy both steroids and immunosuppressive agents might be useful (4)

Congenital macrothrombocytic thrombopenia

The three patients included in this group underwent splenectomy after a provisional diagnosis of idiopathic thrombocytopenic purpura. Studies during the following years revealed that they had a congenital thrombopathia with very large platelets with a pathological pattern when studied by electrophoresis (11) Bleedings had persisted and no increase of the number of platelets had occurred after the operation (Table IV) Some of the patients with idiopathic thrombocytopenic purpura reported in previous studies and showing no response to splenectomy may also belong to this group and this condition should therefore be ruled out before splenectomy is decided upon

Hemolytic Anemia

Hereditary spherocytosis

Of the original 33 patients 25 were examined at follow up one died of apoplexy 14 years after

Table IV *Congenital macrothrombocytic thrombopenia*

Hematological condition before and an average of 8 6 years after splenectomy

	No	Erythrocytes (10⁶ mm)	WBC (mm³)	Platelets (mm)	Hb (g 100 ml)
Before operation	3	4 2 (4 1–4 3)	7500 (5600–10 500)	17 600 (4000–25 000)	13 2 (12.1 14 6)
8 6 (6–11) years after operation	3	4 9 (3 7 5 7)	8900 (7400–9800)	15 000 (8000–21 000)	13 9 (11 1–17 8)

Table V *Hereditary spherocytosis*

Hematological condition before and an average of 8 0 years after splenectomy

	No	Erythrocytes ($\times 10^6/mm^3$)	WBC ($/mm^3$)	Platelets ($/mm^3$)	Hb (g/100 ml)
Before operation	25	3 7 (2 0–5 2)	8700 (1400–36 000)	224 000 (128 000–480 000)	10 9 (3 8–15 ?)
8 0 (3–12) years after operation	25	4 8 (4 0–5 4)	8500 (4500–14 600)	471 000 (182 000–842 000)	14 3 (11 5–18 4)

operation at the age of 58 years and seven could not be traced at the time of follow up

All patients had an enlarged spleen at operation Nine patients or 27 % had gallstones No complications were registered at operation All the patients examined were in excellent condition and had no signs of anemia (Table V) A definite tendency to thrombocytosis was registered Seventy % of the patients had platelet counts higher than 400 000/mm³ but no episodes of thromboembolic complications could be traced

Hereditary spherocytosis is regarded as a definite indication for splenectomy The present study supports this view (8 25) Thrombocytosis frequently follows operative procedures Usually this is a transient reaction immediately following the operation and more pronounced after splenectomy than after other operations (12 21) The pronounced and long lasting thrombocytosis in patients with hereditary spherocytosis shows that the mechanism regulating platelet production and/or destruction is out of balance The position of the reticulo-endothelial system must be considered in this connection and platelet survival studies in these patients could be of interest

Congenital non spherocytic hemolytic anemia

Two patients with a pyruvate kinase deficiency of the red cells had an enlarged spleen at operation No change in their anemia was observed after splenectomy (Table VI)

Lack of spherocytosis a normal osmotic fragility of the red cells as well as enzymatic abnormalities distinguish this group of patients from patients with hereditary spherocytosis No effect of splenectomy has been reported (25) and it is therefore important to exclude this group of patients before splenectomy is considered in congenital hemolytic anemia It should be mentioned that the enlarged spleen by itself might induce symptoms in the course of the disease which may indicate splenectomy

Acquired hemolytic anemia

Eighteen patients with an acquired hemolytic mechanism have undergone splenectomy The anemia had in all cases occurred without known cause The symptoms had been present for an average of 24 months before operation A positive direct Coombs reaction was present in 16 of the patients and two of these had a high level of

Table VI *Hereditary non spherocytic hemolytic anemia*

Hematologic condition before and an average of 8 5 years after splenectomy

	No	Erythrocytes ($\times 10^6/mm^3$)	WBC ($/mm^3$)	Platelets ($/mm^3$)	Hb (g/100 ml)
Before operation	2	2.9 (2 6–3 1)	3100 (2900–3300)	2_6 000 (177 000–275 000)	9 0 (7 4 10 6)
8 5 (3–14) years after operation	2	3 1 (3 0–3 2)	5 00 (4 00–5800)	408 000 (256 000–563 000)	9 8 (9 4–10)

Table VII *Acquired hemolytic anemia*
Hematological condition before and an average of 9 8 years after splenectomy in the surviving 10 patients

	No	Erythrocytes (× 10⁶/mm³)	WBC (/mm³)	Platelets (/mm)	Hb (g/100 ml)
Before operation	10	3 0 (2 2–4 5)	7900 (4000–23 400)	181 000 (57 000–410 000)	9 5 (8 2–12 8)
9 8 (5–14) years after operation	10	4 1 (3 4–4 8)	8800 (3600–18 700)	338 000 (197 000–450 000)	13 6 (12 3–14 6)

circulating cold agglutinins Steroid treatment before operation had been given to 17 patients and of these 14 had a remission However when the dosage was reduced or withdrawn the symptoms reoccurred and the effective dosage was too high for long term therapy (> 10 mg of prednisone per day)

The spleen was usually enlarged (Table II) Two of the patients had gallstones No complications occurred at or shortly after operation

At follow up ten patients were in excellent condition (Table VII) and only one was on continuous steroid treatment Thrombocytosis was not registered in this group of patients Seven patients had died during the observation period an average of 3 2 (1–6) years after splenectomy Four had a severe hemolytic syndrome without response to steroids Of these one had chronic pyelonephritis and one had endocarditis with septicemia The other three patients died of malignant lymphoma, coronary heart disease and a septicemia following operation for bornchiectasis The frequency of positive direct Coombs reaction and the effect of cortisone before operation had no prognostic value The two patients with a high level of cold agglutinins died one and six years after splenectomy of coronary heart disease and malignant lymphoma No effect of splenectomy in patients suffering from the cold agglutinin syndrome can usually be expected (19)

Steroid treatment has been used for many years in patients with acquired hemolytic anemia. Sustained remissions have been few (6) although complete initial relief has been observed in 70–90 % (9) Spontaneous remissions do occur but seldom When no response to steroid treatment is observed or when a high dosage has to be maintained for more than three months splenectomy should be recommended In the present study more than 50 % of these patients were cured after splenectomy Serious complications postoperatively were not observed in any of the patients

Pancytopenia

Thirty five patients with chronic pancytopenia of unknown etiology and without response to medical treatment were subjected to splenectomy The symptoms had been present for an average of 29 months before operation Nineteen patients had a hypoplastic or aplastic bone marrow with a certain degree of lymphocytic infiltration judged by three or more bone marrow aspirations and 16 had normal or increased bone marrow cellularity

At the time of operation nine of the patients had a moderately enlarged spleen

Twenty-six or nine of the patients were in fairly good condition at the time of follow up an average of ten years after the operation

Table VIII *Pancytopenia*
Hematological cond tion before and an a erage of 10 years after splenectomy in the survi ing 9 pati nts

	No	Erythrocytes (10⁶ mm³)	WBC (/mm)	Platelets (/mm)	Hb (g 100 ml)
Before operat on	9	2 7 ([illegible]–3 4)	3100 (1500–5900)	49 000 (8500–150 000)	9 7 (7 9 1[illegible] 9)
10 (7 13) years after operat on	9	3 3 (2.0–4 9)	5700 ([illegible]600–9900)	[illegible]43 000 (35 000–658 000)	11 5 (7 4–14 1)

(Table VIII) Two were on continuous steroid therapy and a third had moderate pancytopenia Six of these patients had a normal or hyper cellular bone marrow before the operation and one of the three with a hypoplastic bone marrow had persistent symptoms of pancytopenia Three patients were not found at the time of follow up Twenty three or 65% of the patients had died Three of them died of heart failure within one week after operation One of these had evidence of a myelogenous leukemia at autopsy whereas no explanation for the pancytopenia was found in the other two

The average survival time of the remaining twenty patients was 36 months and most of these showed some improvement after splenectomy The causes of death were as follows three died of hemosiderosis with heart failure 4–15 years after operation They had needed blood transfusions every 2–3 weeks and their transfusion requirements had only been moderately influenced by splenectomy two died of acute leukemia 4 and 20 months after operation three died of septicemia 2–11 years after operation two died of severe bleeding 3 and 84 months after operation and finally the cause of death was unknown in ten patients

Pancytopenia is the main symptom in a heterogenous group of diseases the nature of which is unknown at the time splenectomy has to be considered The results vary considerably and the response cannot be predicted A normal or hypercellular bone marrow could indicate a splenic marrow depression and thus strengthen the indication for splenectomy It should be stressed that bone marrow biopsy and not only aspiration should be carried out in all cases

Heaton et al (13) reported on the results of splenectomy in 47 cases of hypoplastic or aplastic anemia About 50% appeared to have improved though the observation time of many of these patients was short Three of eight cases reported by Sandusky et al (22) were alive an average of 4 1/4 years after splenectomy one of them with normal hematology Other reports have shown improvement in about 30 per cent of the cases (10 23)

The present study as well as earlier reports indicate that splenectomy might definitely improve the condition of a certain number of patients with pancytopenia particularly when a normal or a hyperplastic bone marrow cellularity is present The operative procedure represents a significant danger in these patients and careful observation and treatment before and after operation is imperative Increasing knowledge on the mechanism leading to pancytopenia might narrow the indications for splenectomy in this group of patients

Proliferative Diseases

Chronic lymphatic leukemia

Nine patients have been subjected to splenectomy seven of them because of a severe hemolytic syndrome associated with red cell sequestration in the enlarged spleen and two because of thrombocytopenia Medical treatment had failed At the time of operation the white cell count was normal or only moderately increased whereas the platelet count usually was decreased At operation an enlarged spleen was found in all cases

Only one of the patients was alive at the follow up 32 months after surgery He showed no evidence of hemolysis and the situation was controlled by medical treatment One patient with an additional aortic valvular disease died two weeks after operation of heart failure The mean survival time of the other seven patients was 18 (5–36) months The hemolysis was reduced in all but one patient and the thrombocytopenia also showed improvement after operation However the hemolytic syndrome as well as the thrombocytopenia again became severe before death

The present study as well as other reports indicate that relief of symptoms not controlled by steroids or antileukemic agents can be obtained by splenectomy (14 24) The remission usually lasts about 18 months Remissions lasting several years however have been observed

Chronic myelogenous leukemia

Five patients have been splenectomized Three of them had severe hemolysis and two thrombocytopenia All medical treatment had failed and the splenectomy was carried out in a desperate situation

All except one had a markedly enlarged spleen None of the patients died as a result of the operative procedure Their symptoms however persisted and the average survival time ranged from 2 to 4 months

Table IX *Reticulosis*

Hematological condition before and an average of 4 7 years after splenectomy in three surviving patients

	No	Erythrocytes (× 10⁶/mm³)	WBC (/mm³)	Platelets (/mm)	Hb (g/100 ml)
Before operation	3	3 5 (2 4–4 4)	4900 (2600–9200)	67 000 (30 000–103 000)	9 1 (7 –10 5)
4 7 (4–5) years after operation	3	4 1 (3 2–4 9)	6000 (3300–9200)	400 000 (320 000–472 000)	12 5 (9 5–15 1)

Even if improvement following splenectomy has been reported in certain instances of chronic myelogenous leukemia (24) the conclusion at present must be that splenectomy is of no value and this disease should rather be considered a contraindication to surgery

Malignant reticulosis

Nine patients including six cases of lymphosarcoma two cases of Hodgkin s disease and one with myelomatosis were subjected to splenectomy The main indication for surgery was abdominal discomfort hemolytic anemia and thrombocytopenia The diagnosis usually was established before operation by histological examination of lymph nodes or aspiration smears from the enlarged spleen

All patients except one with a lymphosarcoma invading the lumbal part of the spinal column had greatly enlarged spleen This patient with a normal-sized spleen and severe thrombocytopenia obtained no remission and succumbed one week after surgery because of bleeding

At the time of follow up three patients were alive (Table IX) Two patients with lymphosarcoma of the spleen and with 60° young lymphoid cells in the blood smears were in good condition with normal hematology 3 and 4 years after surgery respectively One patient with Hodgkin s disease and with a spleen weighing 3000 g improved considerably after operation five years ago The last year she has again had an increasing activity of her disease The indications for splenectomy in these three patients was thrombocytopenia and abdominal discomfort. The remaining five patients died 5 to 15 months after splenectomy which thus induced only a short remission

Although splenectomy generally seems to be of little value in malignant reticulosis marked and sustained improvement has been reported (14 22 24) Our two cases with lymphosarcoma show that even if lymphoid proliferation is present in the bone marrow and young lymphoid cells are present in peripheral blood an excellent remission occurred where steroid and X ray therapy failed to give a significant improvement The extent of the disease and the histology of the spleen seem to give important prognostic information (1 16)

Myelofibrosis

Three patients are included in this group In two of them the disease was preceded by polycythemia 4 and 17 years before splenectomy respectively The indication for surgery was pancytopenia associated with a hemolytic syndrome in two of them and abdominal discomfort no longer responsive to X ray therapy in one

All patients had a markedly enlarged spleen No serious operative complications were registered All patients showed a short lasting improvement The main course of the disease however seemed unaffected and all three died 8 to 14 months after splenectomy

The old concept that myelofibrosis with myeloid metaplasia represents a contraindication to splenectomy does not seem to be valid Splenectomy does not induce further disturbance of the hemopoiesis (3 7 17) When repeated hemorrhages recurrent anemia or the discomfort of splenomegaly cannot be controlled by other procedures splenectomy should be considered Some authors claim that in the absence of contraindications the spleen should be removed as soon as the diagnosis is established The contraindications are related to the patient s general condition and not to the value of the spleen as a hemopoietic organ (7) Our experience is not sufficient to argue with this point of view

Table X *Congestive splenomegaly*

Hematological condition before and an average of 5 3 years after splenectomy

	No	Erythrocytes ($\times 10^6/mm^3$)	WBC ($/mm^3$)	Platelets ($/mm^3$)	Hb (g 100 ml)
Before operation	11	4 1 (3 2–4 9)	3500 (1500–6 00)	36 000 (6000–78 000)	11 4 (8 8 13 6)
5 3 (3–1) years after operation	11	4 4 (3 9–5 0)	6800 (4 00–10 500)	217 000 (79 000–419 000)	13 6 (11 5–15.2)

Felty s Syndrome

Only two patients with this disease have undergone splenectomy Both had symptoms of rheumatoid arthritis for about 20 years and the indication for operation was maturation arrest of the myeloid cells with neutropenia

No complications occurred at operation and both had a markedly enlarged spleen (Table II)

Both patients showed immediate improvement after surgery One patient died eight years later with symptoms of chronic renal failure whereas the long term effect in the other was excellent

Many reports of excellent results on the hematological failure in Felty s syndrome following splenectomy have been published (7 15 25) This syndrome thus seems to represent an indication for splenectomy

Congestive Splenomegaly

Seventeen patients are included in this group (Tables I and X) Seven patients had cirrhosis of the liver five Banti s disease three thrombosis of the splenic vein and finally two had circulatory disturbances caused by Wilson s disease and amyloidosis All patients had symptoms of hypersplenism with thrombocytopenia and neutropenia. This represented in addition to varicose esophagal veins in six patients the main indication for surgery

All patients had an enlarged spleen at operation No serious complications occurred postoperatively

At the time of follow up eleven patients were alive and in good condition One patient with Banti s disease still had thrombocytopenia but no bleeding All the other ten three with Banti s disease all three with thrombosis of the splenic vein three with hepatic cirrhosis and the one with Wilson s disease all showed complete hematologic remission when examined an average of five years after operation The fate of two patients with Banti s disease is unknown Five patients who initially showed a good remission died an average of 30 (2 to 144) months after operation Four of them had a progressive hepatic insufficiency and the fifth died two months after surgery with severe amyloidosis

As far as the hematologic disturbances caused by chronic congestive splenomegaly are concerned splenectomy can be recommended However the disease leading to splenomegaly is often not influenced by this procedure and shunting operations have to be considered In some reports the immediate postoperative mortality has been high and recurrences of hemorrhages have been reported (26)

CONCLUSIONS

The present study includes 179 patients subjected to splenectomy for a variety of hematological disorders Thirty five % of the patients had died eight of these or 4 4 % within six weeks after surgery Eight % of the patients could not be traced at the follow up The known survivors 57 % were examined 3 to 15 years after surgery The anticoagulant treatment given routinely to most patients postoperatively may in part be responsible for the lack of thromboembolic complications

Our own experience and a review of the pertinent literature suggest the following conclusions

1 A lasting remission is obtained in about 75 % of patients with *idiopathic thrombocytopenic purpura* There is no relation between the preoperative effect of steroids and the results following splenectomy Long term treatment with steroids before splenectomy should be abandoned

The use of steroids preoperatively to reduce bleeding complications during operation is questionable Patients with *congenital macrothrombocytic thrombocytopenia* should be excluded from the group of idiopathic thrombocytopenic purpura These patients do not benefit from splenectomy

2 *Hereditary spherocytosis* represents an indication for splenectomy and complete remission might be expected in most cases A persistent thrombocytosis after splenectomy was observed in more than 50% of these cases but this did not cause any complications

3 *Hereditary non spherocytic hemolytic anemia* is not influenced by splenectomy

4 When complete remission of an *acquired hemolytic anemia* has not been obtained after steroid therapy for three months or if the maintenance dosage might induce side effects splenectomy should be recommended More than 50 of these patients showed no symptoms of hemolytic disease when examined ten years after surgery and another 30% showed long lasting improvement Symptoms related to a high titer of cold agglutinins are not influenced by splenectomy

5 When all medical therapy has failed splenectomy should be considered in patients with *pancytopenia* Nine of 35 patients of whom six had a normal or a hypercellular bone marrow were in good condition ten years after splenectomy A normal or increased bone marrow cellularity therefore strengthens the indications for surgery At least 10 of our patients with pancytopenia later died of leukemia

6 Remissions lasting about 18 months can be expected in patients with *chronic lymphatic leukemia* subjected to splenectomy because of a severe hemolytic syndrome or thrombocytopenia which cannot be controlled by other treatment No benefit can usually be expected from splenectomy in *chronic myelogenous leukemia*

7 *Lymphosarcoma* of the spleen represents an indication for surgery The result of the operation depends on the extent of the disease and the histology of the spleen A certain degree of lymphocytosis of the bone marrow and the peripheral blood does not contraindicate splenectomy Two out of six patients were in good condition with normal hematology 3 to 4 years after operation

Occasionally splenectomy might be recommended in *Hodgkin s disease* associated with severe hemolysis and thrombocytopenia

8 *Myelofibrosis* is not a contraindication for splenectomy Patients with repeated hemorrhages recurrent anemia or the discomfort of splenomegaly which cannot be improved by other forms of therapy should be subjected to splenectomy It might be that the indications should be extended The average survival time in our three patients was 12 months

9 *Felty s syndrome* includes a hematological disturbance This part of the syndrome usually shows a significant improvement after splenectomy

10 Thrombocytopenia and neutropenia associated with *congestive splenomegaly* usually can be corrected by splenectomy The disease leading to splenomegaly however determines the post operative course and the requirement for shunting operations has to be considered

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CHOREA MINOR ASSOCIATED WITH SYSTEMIC LUPUS ERYTHEMATOSUS

Report of a Case

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Abstract Chorea minor associated with systemic lupus erythematosus has hitherto been reported in 1_ cases A further case is described in a 28 year-old woman who at the age of 16 had an attack of chorea that lasted three months In the following years she developed an elevated blood pressure a false positive Wassermann s reaction for syphilis and now 1 years after the onset of chorea she has peripheral neurological signs lupus nephritis and positive L E *cell phenomenon*

Systemic lupus erythematosus (SLE) is not unusually associated with symptoms from the central nervous system In a series of 216 patients Jessar (8) found a frequency of 16% for neurologic psychiatric symptoms mainly psychoses convulsions and hemiplegias Only a few cases of chorea in patients with an earlier or later diagnosed SLE have been published In the following such a case is described and a brief review is given of the previously published cases

CASE REPORT

The patient is a now 28 year-old woman who at the age of 1? sustained a head injury followed by vomiting and headache but without loss of consciousness In May 1954 she had pains in her arms and legs. She was hospitalized in August 1954 at the age of 16 Six weeks before admission she had developed facial grimaces and purposeless movements of her hands and feet. On entering the hospital she showed continuous uncoordinated movements of all four extremities trunk head and face Physical examination was otherwise negative Laboratory findings ESR was initially 25 mm/h rose to 45 and fell to 19 at the time of discharge Hb was 102% the leucocyte count was 8300/mm with a normal differential and remained within the normal range on repeated examination The blood pressure was 150/100 mm Hg There were no hemolytic streptococci in the throat culture Antistreptolysin-O titer was normal on repeated examination but on one occasion it was 00 U/ml Antistreptocochyaluronidase titer was on repeated examination within the normal range Chest X ray and electrocardiogram were normal Urine analysis showed no protein or glucose Serum protein electrophoresis was normal

Throughout hospitalization her temperature remained normal She was treated with acetylsalicylic acid and for a fortnight with penicillin Chorea disappeared about the 1st of October having then persisted for three months The patient was discharged in December 1954 in good condition For the following six months she was seen as *an out patient and during this time she was clinically* free from symptoms ESR was continuously elevated between 22 and 30 mm h Hb was 104 Titers of antistreptolysin-O and antistreptocochyaluronidase were within normal limits

Seven years later in 1961 she was admitted to the surgical department of this hospital because of an abortion During hospitalization her blood pressure was found to be elevated with a minimal value of 170/1?0 mm Hg On clinical examination a systolic high frequent rough murmur was heard over the apex of the heart Chest X ray ECG and ophthalmoscopic examination were normal as was the excretion of norepinephrine in urine ESR was 106 mm/h Hb 65–71 serum creatinine 1 3 mg and Wassermann reaction for syphilis negative The patient left the hospital before further investigations could be carried out

In 1964 she was re hospitalized in the surgical department because of another abortion Her Hb was 9? ESR 52 mm/h and the blood pressure varied between _00/130 and 160/130 mm Hg There was no protein or glucose in the urine At this time the Venereal Disease Research Laboratories test and the Wassermann reaction for syphilis gave false positive reactions, while the Treponema pallidum immobilisation test was negative During observation an unexplained exanthema was seen on her neck and trunk

In February 1966 the patient was seen in the medical out patient clinic because of pains in her left forearm for six or seven weeks and transient loss of strength in her left arm and in the left part of her face six weeks before She felt tired and uncomfortable but had not experienced headache dizziness or fever On clinical examination the patient was found to be in good general condition The blood pressure was 30/140 mm Hg There was a slight hypoesthesia of the left zygomatic area and a left sided facial paresis around her mouth In her left upper extremity there was hypoesthesia of the hand and

fingers and hypoalgesia of the fingers. Furthermore there were astereognosis, dysdiadokokinesis and loss of postural sense.

Laboratory investigations were as follows: ESR was consistently elevated between 47 and 70 mm/h. Hb was 14.6 g%, white blood count 7300/mm with a normal differential. Creatinine clearance was about 50 ml/min. The urine contained between 0.3 and 1.7 g of protein daily, while the sediment was normal. Titers of antistreptolysin-O and antistreptococchyaluronidase were normal. The Rose-Waaler and the latex fixation tests were negative, while the antinuclear factor varied between negative and [illegible] positive. On two examinations the Venereal Disease Research Laboratories test was positive while the Wassermann reaction for syphilis and the Treponema pallidum immobilisation test were negative. The L. E. cell phenomenon was positive on three occasions. One of several serum protein electrophoreses showed a slightly elevated gamma globulin concentration. ECG and chest X-ray were normal. Renal aortography showed that the vessels were normal. The renal cortical zone was diminished and there was a rapid venous filling on both sides. The EEG was moderately abnormal, especially over the right hemisphere and maximally over the temporal region, there being 5–7 cycles/sec, often with augmented voltage. At six examinations the EEG remained unchanged for the next three months. Ophthalmoscopy revealed that the arteries were slightly narrowed and of uneven caliber. There were a few cottonwool exudates and small hemorrhages. A renal needle biopsy containing 50 glomeruli showed half of these to be collagenously sclerotic. In the open glomeruli the vascular tuft showed a uniform eosinophilic thickening of the basal membranes, often resulting in the formation of typical wire loops. There was proliferation of the normal cells and focal infiltration with leucocytes. Almost all the arterioles showed a prominent thickening of their walls because of proliferation of the intima.

Clinical course and treatment

The temperature remained normal throughout hospitalization. After four weeks, therapy was started with prednisone initially 60 mg daily, later with diminishing doses. A week afterwards the patient felt subjectively better, but objectively there have been no changes in the neurological phenomena. EEG, renal function, proteinuria and sediment have not altered and the blood pressure has remained high at diastolic values of 1[illegible]0 to 160 mm Hg.

DISCUSSION

Twelve cases of chorea in association with SLE have hitherto been published (1–6, 9–13). A survey of some of these cases has been given by Paradise (11) and Rowe (12). All 13 patients have been women. This fact does not seem surprising as the sex ratio in SLE is usually given as nine females to one male.

The patients have been between nine and 23 years of age, with an average age of 15, at the time of the appearance of the first symptom of SLE. In ten of the 13 cases the disease appeared in the second decade, only three patients experiencing their first symptom after the age of twenty. The youngest patient was ten years old and the oldest 33 when they developed chorea, the average age for the appearance of chorea being 17 years.

The duration of chorea varied from ten days to three years. In some cases there have been free intervals in the course of chorea. The time relation between chorea and SLE shows no characteristic pattern. In seven of the patients chorea appeared before disseminated symptoms with a maximum interval of 12 years, in one patient it occurred simultaneously with other symptoms, and in five patients manifest SLE preceded chorea by as much as ten years.

Other signs of affection of the central nervous system have been present in seven of the patients who suffered from psychoses, epilepsy or peripheral neurological phenomena. EEG was recorded in only three patients and has shown only diffuse abnormalities.

Glaser (5) has described the central nervous pathology in SLE. He found that the basic lesion is a necrotizing arteritis of the small arteries and arterioles with fibrinoid alterations in all vascular layers. These lesions have a predilection for the gray matter of the cerebral cortex while the basal parts of the brain are affected only to a small extent. Besides the primary vascular lesions he found perivascular hemorrhages, tiny cysts and infiltration with lymphocytes and mononuclear phagocytes. In the periphery of the degenerative areas there was an abundance of astrocytes.

Cerebral autopsy has been carried out in four of the 13 cases mentioned. In three of the cases changes were found similar to those described above and with the same localisation. In only one of these three cases was a lesion of the lenticular nucleus described.

In the examined cases of chorea and SLE no specific focal localisation of the cerebral lesions has been found which could account for the association. This is in accordance with the opinion held by many investigators that chorea does not result from damage to any single central nervous system structure, but in the majority of cases is caused by multiple disseminated lesions in the basal ganglia, thalamus, other extrapyramidal

nuclei cerebral cortex cerebellum and their mutual interconnections (7)

Gerok (4) treated his case with prednisone 30 mg daily whereby chorea subsided in ten days Rowe (12) used 20 mg prednisone daily and chorea vanished in four weeks Mercaptopurine in a dose of 150 mg daily for ten days was used by Greenhouse (6) Chorea disappeared two weeks after the discontinuation of the drug because of leucopenia

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CONGENITAL HEART DISEASE IN MIDDLE AGED ADULTS

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Abstract A clinical and haemodynamic survey is given of 112 patients above the age of 40 years with congenital heart diseases studied at a Cardiological Centre in Norway from 1960 to 1965 Atrial septal defect was found in 61 ventricular septal defect in nine patent ductus arteriosus in 16 pulmonic stenosis in 14 coarctation of the aorta in 11 and Ebstein s anomaly of tricuspid valves in one patient The sex distribution was on the whole similar to that of younger groups

The above diagnoses should be kept in mind when dealing with middle aged heart patients Twenty subjects had been previously treated under erroneous diagnoses notably rheumatic pulmonary or coronary heart diseases The reasons were most often the finding of an apical diastolic murmur in patients with left to-right shunt a history of typical angina pectoris which occurred in 11 patients or the presence of atrial fibrillation which altered the auscultatory signs e g in atrial septal defect.

Even if routine health control had led to the diagnosis in many cases there was a high incidence of troublesome symptoms pulmonary hypertension and other complications A comparison of younger and older adults showed a definite worsening of symptoms and complications in the latter group

Surgical treatment was carried out in 73 patients 25 of whom had arrhythmia or failure before the operation Six died in the postoperative period but most patients showed definite improvement.

The knowledge of the natural history in congenital heart diseases is incomplete Information about symptoms and signs is especially sparce concerning adults in the older age groups Most clinical materials even from large centres comprise only a few patients above the age of 40 There may be several reasons for this 1 Perhaps most patients die earlier 2 Symptoms and signs may change during the ageing process and correct diagnosis may not be made frequently enough 3 Perhaps many patients are free from complaints or ascribe symptoms such as tiredness and dyspnoea to age and do not consult a doctor

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MATERIAL

The following report presents a group of patients with congenital heart disease examined at a Cardiological Laboratory from 1960 to 1965 During this time most patients with congenital heart disease who were operated on in Norway passed through this particular unit In many cases routine health control had led to the diagnosis The description mainly concentrates on the group above the age of 40 years comprising 112 patients With one exception the diagnosis was verified by hemodynamic examination and/or operation The exception was a patient with Ebstein s anomaly of the tricuspid valves in which condition heart catheterization carries a high risk of complications (23) Patients with aortic stenosis are not included as it is often difficult to tell by clinical and haemodynamic examination whether the stenosis is congenital or acquired Other conditions excluded are Marfan s syndrome primary pulmonary hypertension and familial cardiomyopathy

RESULTS

Atrial septal defect was by far the most frequent anomaly (Table I) Most patients were between 40 and 60 years old and only three were older The sex distribution was about the same as in younger age groups women being predominant among patients having atrial septal defect or patent ductus and men among patients having coarctation of the aorta while pulmonic valve stenosis and ventricular septal defect are more evenly distributed between the sexes (12)

Atrial septal defect

The preponderance of atrial septal defect in patients above the age of 40 years is in agreement with previous smaller materials (11 17) This anomaly is therefore described in more detail than the others and a comparison is made between younger and older adults Table II shows that the proportion of men was roughly similar above and below 40 years of age Campbell and Neill (5) found that the proportion of men was

Table I *Congenital heart disease in patients over 40 years of age*

Lesion	No	Age range (y)	Sex ♂	Sex ♀
Atrial septal defect	61	40–68	17	44
Ventricular septal defect	9	40–59	6	3
Patent ductus arterio us	16	40–59	3	13
Pulmonic valvular stenosis	14	40–59	9	5
Coarctation of the aorta	11	40–56	9	2
Ebstein s anomaly	1	49	1	0
Total	112		45	67

less in the older age groups This has not been corroborated by the present material comprising a larger number of patients In the age group 20 to 40 years 17 patients had defects of the primum type and 67 defects of the secundum type The corresponding figures above 40 years of age were 2 and 59 This emphasizes the poor prognosis in primum defect Nearly all patients above 40 years of age had symptoms as a rule dyspnoea on exertion but also chronic bronchitis or pal pitations Three patients had typical angina pec toris and repeated loss of consciousness was the main symptom in two others Table III shows the grouping in classes according to the New York Heart Association Nomenclature More than half the patients between 20 and 30 one third of the patients between 30 and 40 but only 4 of 61 above the age of 40 years were in class I More than half of those above 40 had symptoms dur ing relatively slight exertion However 15 of them were diagnosed after routine X ray screening or other health control On the other hand at least five patients some with marked symptoms and large defects had passed one or more screenings without being notified

Table IV shows that arrhythmia occurred rarely in younger adults but was more frequent in older

Table II *Atrial septal defect in adults*

Age and sex

Age	♀	♂
20–40	56	28 (33)
40–60	41	17 (29)
>60	3	0
Total	100	45 (31)

Table III *Atrial septal defect in adults*

Functional classification

Age	I	II	III	IV
20–30	28	16	4	0
30–40	3	22	5	0
>40	4	26	27	4

age groups Among patients above the age of 50 years more than one third had atrial fibrillation or flutter Various complications occurred in more than half the patients above 40 years of age Besides the disturbances of rhythm in 17 clear signs of right sided congestive heart failure were present in eight patients and in five others there was information from the patient himself or from previous admissions of periods with oedema and enlarged liver Four patients had attacks of dyspnoea at night Among these two had angina pectoris or hypertension but in the other two no other symptoms or signs of left sided heart affection or mitral stenosis were found Lung oedema in atrial septal defect has previously been described (5) but is difficult to interpret because haemodynamically there is over loading of the right ventricle and no extra load on the left ventricle A possible explanation is paroxysmal tachycardia or atrial fibrillation In the three patients with angina pectoris the pain had a typical localization was typically related to exertion and was relieved by nitroglycerin Systemic embolies had occurred in three patients two with atrial fibrillation and one with sinus rhythm

Only one of the 61 had mitral stenosis which was verified on operation This emphasizes that Lutembacher s syndrome probably occurs less fre

Table IV *Atrial septal defect in adults*

Arrhythmia

Age	Atrial flutter/ fibrillation	Total no of pats
20–30	1	48
30–40	2	36
40–50	7	33
50–60	9	25
>60	1	3

Table V *Atrial septal defect in adults*
Systolic pulmonary artery pressure (mm Hg)

Age	Pressure over 35	Pressure over 70	Total no
20–30	6 (13)	3 (6 %)	48
30–40	8 (22)	3 (8)	36
> 40	26 (43)	8 (13)	61

quently than previously believed None of the patients with atrial septal defect had had endocarditis

The clinical diagnosis of atrial septal defect is usually simple (2) On auscultation a relatively weak systolic murmur is found in the second and third left intercostal space The second sound in this area shows fixed splitting The electrocardiogram shows incomplete right bundle branch block and on heart X ray the right ventricle and the pulmonary artery are large while the aorta is usually small Most patients above the age of 40 years had these classical signs On the other hand 13 of the 61 patients had previously been treated under other diagnoses especially rheumatic valvular disease cor pulmonale coronary or arteriosclerotic heart disease Several factors may complicate the diagnosis in the older age groups In atrial fibrillation the splitting of the second sound in the pulmonary area is not constant but varies with the length of the previous diastole A diastolic murmur at the apex is sometimes heard in patients with atrial septal defect The left atrium is usually of normal size but in 16 of the 61 patients above the age of 40 years the left atrium was roentgenologically described as enlarged

As regards the haemodynamic findings there was a definite increase in the incidence of pulmonary hypertension in the older age groups The reference level for pressure recordings in our laboratory is a horizontal plane through the anterior axillary line (20)

Systolic pressure above 35 mm Hg in the pulmonary artery is considered to be definite pulmonary hypertension Table V shows that the proportion of patients with pulmonary hypertension increased from 12 % in the age group 20 to 30 years to 42 % among patients above 40 years of age There was also a relative increase in number of patients with severe pulmonary hypertension There is thus reason to believe that the pulmonary artery pressure in patients with atrial septal defect increases with age Fig 1 shows that the progression may occur fairly rapidly In a woman aged 51 years at the first examination the pulmonary artery pressure showed a severe increase during the course of three years Sometimes there is a systolic pressure gradient across the pulmonic valve possibly as an expression of relative pulmonic stenosis due to the enlarged blood flow Such a gradient occurred less frequently in patients above 40 years of age than in younger adults The proportion of patients having a gradient of more than 10 mm Hg was 7/61 among the former and 23/84 among the latter The ostium may dilate in the course of time But signs of relative pulmonic valve incompetence would then be expected to occur more frequently in the older age groups This was not the case In the present material and as previously shown (7) a diastolic murmur along the left sternal border was more frequently found in the younger

FEMALE WITH ATRIAL SEPTAL DEFECT

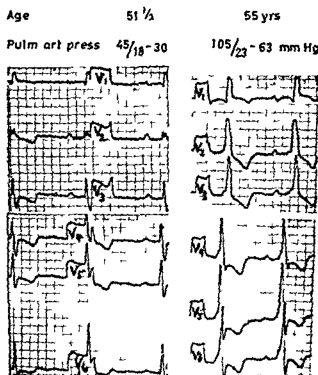


Fig 1 Progression of right ventricular hypertrophy with increasing pulmonary hypertension

Table VI *Atrial septal defect in patients over 40 years of age*

Cases with arterial oxygen unsaturation

Oxygen satur ( )	Syst pulm art press (mm Hg)	Congestive heart failure
90	72	+
90	37	–
89	33	–
89	42	–
89	66	+
88	76	–
88	56	–
88	45	–
82	90	–
65	105	–

age groups Another explanation could be that the shunt was larger in the younger patients with a larger blood flow through the pulmonic ostium There was however no relationship between the size of the shunt and the pressure gradient Other possibilities are a worse prognosis for the patients having a pressure gradient or that the systolic murmur is stronger so that the defect is more easily diagnosed and more often operated on before the age of 40 years

Some patients with atrial septal defect have reduced arterial oxygen saturation and cyanosis which can sometimes be difficult to interpret Table VI shows data for the ten patients above the age of 40 who had an oxygen saturation of 90° or less It is noted that the hypoxaemia is not always an expression of reversed shunt due to a pulmonary artery pressure at systemic level or right sided heart failure The table shows examples of only moderately elevated pulmonary artery pressure combined with hypoxaemia with out signs of congestive heart failure Ventilatory and blood gas measurements showed no alveolar hypoventilation which could explain the hypox aemia One reason could be that the pressure in the right atrium was higher than in the left for short periods of the heart cycle which we were occasionally able to measure Another possibility is partial drainage of the inferior caval vein into the left atrium Such an anomaly may on rare occasions occur after the closure of an atrial septal defect. Postoperative central cyanosis may signify that the anatomy has been changed in such a way that the inferior caval vein at least

Table VII *Atrial septal defect in adults*

Operative mortality

Age	No of pats		Deaths	
	Primum	Secundum	Primum	Secundum
20–40	16	55	3	0
>40	2	37	1	2

partly drains into the left atrium The patient must then be reoperated Such a course was noted in one woman aged 40 years in the present ma terial Of practical interest was that the cyanosis developed gradually from barely noticeable on the 10th day to deep blue colour on the 14th day

The postoperative mortality is seen in Table VII One fifth of the patients with primum defect died In secundum defects the mortality was much less only two out of 92 patients both being above 40 years of age The observation period is still too short to judge the long term results However in the great majority of the patients there was significant subjective and objective improvement after a few months

Some patients with atrial septal defect may reach a high age with few symptoms (16 19) The present survey however shows that severe incapacity is frequently found above the age of forty years Many patients do not live so long Based on autopsy materials the average age at death of patients with atrial septal defect is be tween thirty six and thirty nine years (5 7) The operative mortality in secundum defect is least in young patients but still small in the older age groups Ellis et al (8) report successful operative correction in all their five patients above sixty years of age As there is apparently no definite correlation between the size of the defect and the course or severity of symptoms the conclusion is that patients with atrial septal defect probably should be operated on while they are young but the operative mortality in secundum defect in older adults is not so large that age alone repre sents any contraindication within reasonable li mits

Ventricular septal defect

Eight patients above forty years of age had iso lated ventricular septal defect while a ninth had

in addition aortic valve incompetence While isolated ventricular septal defect in children is about as frequent as atrial septal defect the former is much more rare among adults In a survey of 415 cases found in five hospitals in USA in the course of 12 years Walker et al (22) found only two patients above forty years of age Mark and Young (17) and Griffiths et al (13) found six and three patients respectively with isolated ventricular septal defect in the same age group in the course of twenty to thirty years According to Sandøe (21) the total number of published cases of isolated ventricular septal defect in patients above 45 years of age was probably below 20 The number in the present paper was five

Only one of our patients was symptomless and this was also the only patient who had had endocarditis The others were more or less distressed and more than half were in functional class III or IV Atrial fibrillation was present in four patients A fifth had previously had atrial fibrillation but reverted to sinus rhythm after treatment with quinidine Four patients had congestive heart failure and six had either failure or fibrillation

The reason why ventricular septal defect is so seldom diagnosed in adults is unknown The defect is also rarely found in autopsy materials

However a small defect may easily be overlooked at autopsy if not specially looked for It may be that patients with this defect die before they reach the age of forty years or the defect may often close spontaneously Spontaneous closure has frequently been demonstrated recently (1 18) but most often in infants A year ago we had the opportunity of examining a woman who at the ages of eight and sixteen had been catheterized and a ventricular septal defect with significant left to right shunt had been demonstrated on both occasions When she now was re-examined at eighteen years of age there was no demonstrable shunt even with the sensitive hydrogen electrode Indirect anatomical evidence also suggests that spontaneous closure may occur in older age groups (3) The present material suggests another factor which may partly explain the rarity of the diagnosis in adults In children the diagnosis is as a rule simple with a typical pansystolic murmur in the 4th or 5th left intercostal space In the patients with severe pulmonary hypertension the systolic murmur becomes shorter of

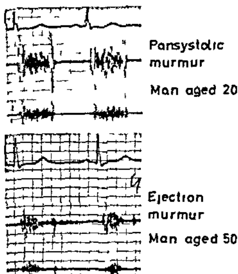


Fig 2 Phonocardiograms recorded from the 4th left intercostal space

the ejection type Among 93 presumably younger patients examined by Hollman et al (14) a short ejection murmur was never connected with a low pulmonary artery pressure Among nine patients above 40 years of age in the present material three had a short ejection murmur in the 4th left intercostal space and two of these had normal or only slightly elevated pulmonary artery pressure An example is shown in Fig 2 It is thus possible that some cases with ventricular septal defect are not diagnosed because the murmur becomes less conspicuous in elderly patients Chest X ray shows enlargement of the left atrium and this together with a diastolic murmur sometimes heard at the apex in addition to the systolic murmur as well as atrial fibrillation was probably the reason for three of the nine patients being treated for shorter or longer periods under other diagnoses especially rheumatic heart disease

As regards the haemodynamic findings only three patients had normal pulmonary artery pressure three had slight or moderate and three severe pulmonary hypertension In comparison 16 of 24 patients aged 20 to 30 years during the same period had normal pulmonary artery pressure With reservation for the small number there were thus as regards this anomaly as well relatively more patients with pulmonary hyper

tension in older than in younger adults. The shunt was demonstrable by means of oxygen gas analysis in eight of the nine patients above 40 but in the 9th only by the more sensitive hydrogen electrode method.

Only one of the nine patients was operated on, but died shortly afterwards. This was the one who in addition had aortic incompetence

Patent ductus

Patent ductus was diagnosed in 16 patients above the age of 40 years. As was the case with atrial septal defect there are reports of individual patients with patent ductus reaching an age of 70 or 80 years (4) Five of the present patients had no symptoms, while the other eleven had varying degrees of dyspnoea on exertion and three had angina pectoris. Six patients had atrial fibrillation or congestive heart failure. The typical continuous murmur below the left clavicula was heard in fifteen patients. In the 16th, with systolic murmur only there was a severe pulmonary hypertension. An enlarged systemic pulse pressure (above 70 mm Hg) was found in eight, and a low diastolic pressure (below 70 mm Hg) in four patients The electrocardiogram showed as a rule diastolic overload of the left ventricle but right ventricular hypertrophy in two patients, left bundle branch block in two and ischaemic changes in one Altogether ten patients had either atrial fibrillation, retrosternal pain on exertion, left bundle branch block or ischaemic changes. Unfortunately we were able to perform coronary angiocardiography on only three of them. In none of these were any pathological changes shown. Also Dailey et al. (6) found that angina pectoris and even a history typical of infarction, were frequent in patients with patent ductus. Autopsy however never showed significant coronary artery disease or myocardial infarction.

Haemodynamic measurements were made in 11 patients and in five of them there was moderate or severe pulmonary hypertension. It is not possible to make a comparison with results in younger age groups as heart catheterization was done more frequently in older age groups and especially when clinical signs of pulmonary hypertension were present. Progressive increase in pulmonary vascular resistance has, however been reported also in patent ductus on serial catheterizations in individual patients (15)

Thirteen of the patients were operated on and survived. Recanalization occurred in two of them. Both were reoperated but one died immediately afterwards

Pulmonic valve stenosis

Among the 14 patients in this group eight had isolated stenosis while four had ventricular septal defect and two atrial septal defect in addition. That pulmonic stenosis is fairly rare in older age groups was emphasized by Fabricius (10) Mark and Young (17) and Engle et al (9) who found none one and seven patients respectively above the age of 40

Two had had endocarditis all had symptoms which in more than half were severe while three had angina pectoris and two congestive heart failure and cyanosis All patients had a loud systolic murmur typically localized in the 2nd left intercostal space and typically diamond shaped on the phonocardiogram while the electrocardiogram showed systolic overload of the right ventricle The only difference from younger patients was that the R wave in V_1 was on the average 7 mm lower than in 14 patients between 20 and 30 years of age with comparable right ventricular pressures but otherwise unselected. All had sinus rhythm. The pressure gradient across the pulmonic valve was less than 50 mm Hg in four between 50 and 100 in six and more then 100 in four patients This is in agreement with Fabricius (10) who found that severe pulmonic stenosis was mostly found in children and younger adults.

It is reasonable to believe that these patients as a rule die before they reach the age of 40 years of age if they are not operated on. Also as regards this anomaly we observed a case with severe progression of the disease A woman aged 52 years had a right ventricular pressure of 79 mm Hg (Fig. 3) On re-examination 2–3 years later the symptoms had increased significantly and the pressure in the right ventricle was now 180 mm Hg.

Eleven of the patients were operated on, as a rule with significant improvement. The operative mortality was zero

Coarctation of the aorta

Coarctation of the aorta was found in 11 of the 112 patients above 40 years of age Four had no

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

Table X. *Congenital heart disease in patients over 40 years of age*

	No. of pats.
Operated	73
Congestive heart failure before operation	16
Arrhythmia or failure before operation	25
Postoperative deaths	6

symptoms attributed to age, overwork, etc. It is noted that only 14 in the whole material had no symptoms, and that more than half were severely distressed. Ten per cent had angina pectoris which, however, sometimes seemed to be provoked by congenital heart diseases. Chest pain on exertion often disappeared after operation. Only four had had endocarditis. It is further shown that cyanosis and finger-clubbing, classical signs in so many congenital heart diseases in children, were relatively rare in older adults.

Almost one third of the whole material had arrhythmia or congestive heart failure, both being poorer prognostic signs than in, e.g., rheumatic heart failure.

Table IX sums up the haemodynamic findings in all patients with shunt. It is noted that about fifty per cent had pulmonary hypertension.

Table X shows that six of 73 patients died in connection with operative treatment. This is a relatively low risk when it is taken into consideration that 25 of the 73 had arrhythmia or congestive heart failure before operation.

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PLASMA AMINO ACIDS IN PREPSYCHOTIC ALCOHOLICS

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Abstract The plasma levels of individual amino acids, amino nitrogen, and ammonia were determined in prepsychotic alcoholics. Low taurine and high glutamic acid levels were the only abnormalities observed A reduction of the plasma amino acid levels previously reported, could thus not be confirmed. The possible significance of the elevated plasma glutamic acid level, which might reflect a deterioration of the cerebral metabolism, is discussed.

Prigot et al (11) reported a reduction of plasma amino acids in incipient delirium tremens. They found that intravenous infusion of amino acids had a favourable effect on the course of delirium tremens and they considered this treatment superior to other treatments previously used. However this investigation was later on criticized by Kalant (8) Other investigators (2 3) reported good results in treating alcohol psychosis with intravenous administration of glutamic acid and arginine.

The purpose of the present study was to examine whether there were such changes in the plasma amino acids in prepsychotic alcoholics that these changes might be of causal significance for the development of the alcohol psychosis

MATERIAL

The material included 30 patients, most of whom had been hospitalized for seven days. All were gravely advanced alcoholics, who had been subject to alcoholic abuse more or less regularly for 8 to 25 years.

Before their admission into the ward they had been drinking excessive quantities of alcohol continuously for several weeks. The intake of food and especially of protein and vitamins had no doubt been very insufficient during this time They were hospitalized because the risk of their developing alcohol psychosis was considered imminent. All the patients showed signs of liver damage in the form of raised levels of transaminases and or enlargement of the liver The treatment, which started immediately after their admission, included large doses of sedatives (alimemazine chlorprothixen chloride chlomethiazole) supplied with injections of thiamine lactoflavine nicotinamide pyridoxine chloride and pantothenic acid. They were on an ordinary diet.

The investigation comprised the determination of plasma amino nitrogen, individual free plasma amino acids and plasma ammonia.

The study was begun with determinations of total plasma amino nitrogen in a group of 12 patients. Of these patients four had earlier been treated in a closed institution for alcoholics, four had been treated in a mental hospital and four had earlier been under care in a convalescent home for alcoholics. One of them died of liver cirrhosis two years after the investigation. None of the patients developed full delirium, but three of them had hallucinations during the first days of treatment.

The individual free plasma amino acids were determined in another group of six patients. Two of these patients had delirium on the third day after their admission and were therefore transferred to a mental hospital Three of them had hallucinations but they never developed complete psychosis. Four had earlier been under care in a mental hospital and three had been treated in an alcoholic institution.

In a third group of 12 patients the plasma ammonia level was determined Four of them had previously been treated in a mental hospital five in an alcoholic institution and two in a convalescent home for alcoholics. Five of the 12 patients had hallucinations during the first days of treatment.

The three groups were similar in regard to the duration and the severity of the abuse of alcohol.

METHODS

Blood sampling was performed in the morning after 12 hours of fasting. The first blood sample was obtained on the day after admission. The blood was collected in heparinized tubes which were immediately centrifuged The amino nitrogen determination was performed within a few hours after the sampling and the determination of free amino acids within two weeks. The plasma had been kept frozen until the day of analysis. The ammonia determination was started within 20 min after the blood sampling. Amino nitrogen was determined by a photo-

Table X *Congenital heart disease in patients over 40 years of age*

	No of pats
Operated	73
Congestive heart failure before operation	16
Arrhythmia or failure before operation	25
Postoperative deaths	6

symptoms attributed to age overwork etc It is noted that only 14 in the whole material had no symptoms and that more than half were severely distressed Ten per cent had angina pectoris which however sometimes seemed to be provoked by congenital heart diseases Chest pain on exertion often disappeared after operation Only four had had endocarditis It is further shown that cyanosis and finger-clubbing classical signs in so many congenital heart diseases in children were relatively rare in older adults

Almost one third of the whole material had arrhythmia or congestive heart failure both being poorer prognostic signs than in e g rheumatic heart failure

Table IX sums up the haemodynamic findings in all patients with shunt It is noted that about fifty per cent had pulmonary hypertension

Table X shows that six of 73 patients died in connection with operative treatment This is a relatively low risk when it is taken into consideration that 25 of the 73 had arrhythmia or congestive heart failure before operation

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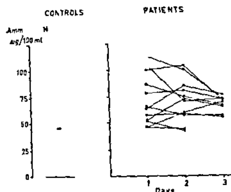


Fig 3 Fasting plasma level of ammonia in 11 healthy subjects and 12 prepsychotic alcoholics on the day after their admission to the ward on the following two days

Plasma ammonia

The plasma ammonia level was essentially normal (Fig 3) In three of the patients the ammonia level was higher on the first day than on the third day after their admission.

DISCUSSION

In alcoholics the amino acid metabolism may be influenced both by the nutritional status and by the abuse of alcohol per se In adults total starvation for short periods induces an elevation of the plasma levels of the branched chain amino acids (18) whereas a diet low in protein produces a gradual decrease in all the essential amino acids (17) Although it is impossible to get any reliable information about the dietary habits of alcoholics most of the subjects studied must no doubt have been in a poor nutritional condition Apart from a low taurine level which could be assigned to protein malnutrition no changes in the plasma amino acids were observed The low taurine level might reflect a low supply of the sulphur-containing amino acids but may also be due to pyridoxine deficiency (16)

It is well known that the abuse of alcohol causes liver damage This seems not only to be due to an inadequate supply of essential nutritives but also to a direct toxic effect on the liver metabolism Alcohol per se results in an increased fatty acid synthesis a reduction of pyruvate to lactate a decreased citric acid cycle activity a decreased gluconeogenesis and disturbances in the catecholamine metabolism (10) These disturbances seem mainly to depend on an increase in the NADH /NAD ratio following ethanol administration

Previous studies (7 11) might also suggest a deterioration of the amino acid metabolism in alcoholics However the low amino acid levels observed in prepsychotic individuals (11) could not be confirmed in the present study A high glutamic acid level was the only obvious abnormality observed It is not known to what extent the plasma level reflects the intracellular concentration of this amino acid The intracellular concentration of glutamic acid is considerably higher than the concentration in plasma. In brain the concentration is remarkably high about 100 times higher than in plasma (4)

The glutamic acid has a central position in the metabolism also in the central nervous system (19), where it has two main functions to act as a precursor of α ketoglutaric acid and to act as a receptor for ammonia (5 20) It is the only amino acid that can be oxidized in the brain (9) The synthesis of glutamic acid and glutamine provides the only two mechanisms for the removal and fixation of ammonia in nervous tissues (4) In alcoholics with deteriorated liver function the removal of ammonia might be reduced In our patients however the plasma ammonia level was not obviously increased although other investigators have reported raised values of plasma ammonia in alcoholics with liver damage (1) especially after physical activity (6) Hakkinen and Kulonen (7) found an increase in the ammonia bound in glutamic acid γ aminobutyric acid and glutamine and a decrease in α-ketoglutaric acid in brain during ethanol intoxication The elevated plasma glutamic acid in [illegible] subjects might reflect a disturbed α ketoglutaric acid-glutamic acid-glutamine balance [illegible] might imply a reduced supply of α ketoglutaric acid [illegible] in the brain resulting in a lowered cerebral respiration

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THE FATTY ACID COMPOSITION IN SERUM OF NORWEGIAN VEGETARIANS

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Abstract The fatty acid composition of cholesterol esters, triglycerides, and phospholipids was determined in healthy vegetarian and non vegetarian men 40 to 70 years of age

Statistically significant differences were demonstrated with high linoleic and low oleic and palmitoleic acid percentages in the vegetarian group in all lipid fractions Differences in palmitic and stearic acid were less consistent but tended to follow those in oleic and palmitoleic acid The differences were more marked in the cholesterol esters and the triglycerides than they were in the phospholipid fraction No differences in arachidonic acid values were observed

A dietary survey performed in the vegetarian group 6 to 1[illegible] months prior to the study revealed high intakes of polyunsaturated fatty acids It is concluded that the study of fatty acid composition in serum lipids has given an indirect confirmation of the findings regarding fat intake in the dietary survey In the vegetarian group statistically significant coefficients of correlation were demonstrated between the dietary intake of polyunsaturated fatty acids in per cent of calories and the percentages of linoleic acid in the cholesterol esters and triglycerides of serum

When absolute values rather than percentage composition were considered it was evident that the main differences between the two groups were those of higher concentrations of some monounsaturated and saturated fatty acids in the control group while the concentration of linoleic acid appeared similar in the two groups The combined effect of polyunsaturated fatty acid intake on the fatty acid composition and on the serum lipid levels offers a reasonable explanation for this finding

Studies of serum lipids in Norwegian lactovegetarians have revealed low values in comparison with values found in subjects on conventional diets (11 12) In the present investigation determinations of the fatty acid composition in serum have been performed in vegetarians and non vegetarians to learn whether the differences in cholesterol triglycerides and phospholipids were accompanied by differences in the fatty acids esterified in these lipid fractions

The previous studies included a dietary survey of the vegetarian group which was found to consume a moderately low fat diet relatively rich in polyunsaturated fatty acids Significant relationships between the dietary fats and the serum lipids were demonstrated However many uncertain factors are involved in a dietary survey based on questionnaires and interviews for the collection of data and on food composition tables for the calculation Since the type of dietary fat was believed to be the most important factor causing the low serum lipid levels a confirmation of the findings regarding fat intake was desirable

Studies of the fatty acid pattern of serum offer a possibility for a more objective evaluation of dietary habits and have been proposed as a guide to the fatty acid intake (6 9 15)

The study therefore tests the validity of the previous dietary survey in the vegetarian group with regard to fat intake

MATERIAL AND METHODS

The study comprised [illegible]1 subjects who had lived on a lacto-vegetarian diet for at least 10 years and a control group of 20 non vegetarians Both groups consisted of healthy men aged 40 to 70 years picked at random among subjects participating also in an investigation published earlier (12)

The dietary data from the [illegible]1 vegetarians in the study revealed a mean intake of 11 33 and [illegible]6 of calories as proteins fats and carbohydrates respectively and a total caloric intake of [illegible]3 8 The proportions of saturated monounsaturated and polyunsaturated fatty acids were 11 11 and 10 of calories respectively The present study was performed 6 to 1[illegible] months after the termination of the dietary survey

Blood was drawn in the morning following a 12 h fast For the fatty acid analysis total lipid extracts of the serum were prepared with a chloroform/methanol mixture (2/1 v/v)

The separation of cholesterol esters triglycerides and phospholipids was performed by column chromatography (7 8 14 17)

Silicic acid Mallinckrodt 100 mesh was first suspended twice in methanol and twice in diethyl ether to remove the smallest particles then dried in air and activated for 20 h at 110 C

Separate columns were prepared for the neutral lipids and for the phospholipids For the former 1 0 g of the silicic acid was suspended in diethyl ether and transferred to the column. The washing of the column was performed with 10 ml of a diethyl ether/acetone mixture (1/1 v/v) followed by 10 ml diethyl ether and finally by 100 ml petroleum ether (b p 60 to 80 C) The total lipid extract from 1 0 ml of serum was dissolved in a small volume of petroleum ether and pipetted to the column Elution of the cholesterol esters was done with 130 ml 1 diethyl ether in petroleum ether and of triglycerides with 100 ml 4° diethyl ether in petroleum ether

For the separation of phospholipids 0 5 g of the silicic acid was suspended in chloroform and transferred to the column The washing procedure included the use of 5 ml of a chloroform/methanol mixture (1/1 v/v) and 10 ml of chloroform. Total lipid extract from 1 0 ml of serum was dissolved in a small volume of chloroform and pipetted to the column The neutral fraction was eluted with 70 ml of chloroform Following this the elution of phospholipids was done with 50 ml of a methanol/distilled water mixture (98/2 v/v)

Aliquots of the fractions were pipetted off for analysis of total fatty acids The hydrolysis of these aliquots was performed according to the method of Pikaar and Fernandes (17) and the titration of fatty acids according to a modification of the method of Dole (4)

The purity of the fractions was tested by thin layer chromatography (TLC) The plates were coated with a layer of Silica Gel H (Merck Darmstadt) washed in a solvent system composed of petroleum ether and ethyl acetate (90/10 v/v) dried in air and activated at 120 C for 1 h. Solutions of the lipid fractions in chloroform were applied to the plate and the TLC was performed in the solvent system described above The plates were afterwards dried in air and sprayed with a 10 solution of phosphomolybdenic acid (Merck Darmstadt) in 96 of redistilled ethanol The lipid fractions were visible after 10 to 15 min at 150 C

Following the column chromatography the lipid fractions were hydrolysed and methylated according to the method of Stoffel et al. (22)

For gas chromatography the methylated fatty acids were dissolved in 0 2 ml n hexane and 1 μl was injected with a microsyringe The apparatus was a Perkin Elmer 880 with flame ionization detector The stationary phase in the column was Chromosorb W 60–80 mesh washed and silanized. The liquid phase was 5 butandiol succinate polyester Highly purified nitrogen with a flow rate of 30 ml/min was used as carrier gas. The column had a length of 2 m and an inside diameter of /₈" The temperature of the column was 195 C of the injection block 270–280 C and of the detector cell 230–240 C For recording of the gas-chromatographic pattern a 1 mV Leed & Northrup recorder with a paper speed of / " per minute was used

The identification of the fatty acid peaks was made by comparison with known standards and by comparison of the relative retention times with the data of other authors (5 15)

The calculation of the fatty acid composition from the gas-chromatographic patterns was made by multiplication of the retention time by the peak height both measured in mm on the recording paper The products for all fatty acids were added and the percentages calculated for the individual fatty acids This procedure is based on the fact that peak widths are linearly correlated to retention times The validity of the method has recently been demonstrated by comparisons with a triangulation procedure (⁹)

The performance of the gas-chromatography apparatus was tested with fatty acid standards obtained from the National Institute of Health USA (Metabolism Study Section Standard Mixtures A B C and D) and showed very good agreement with known values

Recovery experiments were made by adding known amounts of cholesterol palmitate and tripalmitin to the total lipid extracts of serum Following column chromatography hydrolysis and titration of fatty acids 9 of the added cholesterol palmitate was found in the cholesterol ester fraction 91% of the added tripalmitin in the triglyceride fraction (mean of three experiments) The recovery of the methylation procedure was quantitative

The reproducibility of the methods was tested by duplicate determinations in five sera The duplicates were run separately during the whole procedure The results of these studies for the titration of fatty acids and for the gas-chromatographic analysis are recorded in Table I It appears that the results of the gas chromatography have a very good reproducibility for all important fatty acids The reproducibility of very small

Table I *Reproducibility studies standard error of observation in per cent of mean*

Fatty acids	Cholesterol esters	Triglycerides	Phospholipids
Myristic	—	2 5	—
Palmitic	2 0	1 9	4 0
Palmitoleic	4 5	1 5	8 6
Stearic	8 4	3 3	4 3
Oleic	1 5	2 3	1 9
Linoleic	1 0	2 1	2 7
Linolenic	—	8 9	—
Eicosatrienoic	—	—	4 8
Arachidonic	1 6	14 6	4 2
Erucic	—	—	15 0
Docosahexaenoic	—	—	5 6
Total fatty acids	7 8	7 8	2 4

Table II *Cholesterol esters Fatty acid composition and total fatty acids*

Fatty acids,	Vegetarians Mean	S.D	Range	Control group Mean	S D	Range	Result of statistical analysis
Palmitic	9 9	1 4	7 0–12 1	10 7	1 5	8 5–13 5	$0\ 1 > p > 0\ 05$
Palmitoleic	2 4	0 9	1 0– 5 0	3 3	0 9	[illegible]1– 5 0	$p < 0\ 01$
Stearic	1 3	0 7	0 6– 3 2	1 9	0 7	1 0– 3 [illegible]	$p < 0\ 05$
Oleic	14 2	2 7	7 6–19 7	18 7	2 9	12 7–[illegible]5 7	$p < 0\ 001$
Linoleic	61 6	6 4	40 4–68 0	53 3	5 3	45 7–63 8	$p < 0\ 001$
Arachidonic	4 2	1 4	2 3– 6 4	4 3	1 0	2 8– 6 4	$p\ \ 0\ 1$
Eicosapentaenoic?	1 8	1 5	0– 5 2	1 7	1 2	0– 4 3	$p > 0\ 1$
Total fatty acids (mg/100 ml)	67 8	10 6	54 5–94 8	85 5	18 3	49 7–120 7	$p < 0\ 01$

fractions is less favourable and values which are 1 or lower in both groups have therefore been omitted from the tables and are regarded as trace amounts

RESULTS

Tables II III and IV compare the percentage composition of the individual fatty acids and the absolute total fatty acid levels of cholesterol esters triglycerides and phospholipids for the vegetarian and the control group The data are recorded as mean standard deviation (S D) and range

In all fractions the linoleic acid percentages were higher in the vegetarian group The difference was great in the cholesterol ester fraction with mean values of 61 6 and 53 3 respectively and in the triglyceride fraction with 22 3 and 12 8 respectively The difference was less marked in the phospholipids with mean percentages of 27 0 and 22 5 respectively

The differences in linoleic acid percentages were balanced by higher values of some saturated and monounsaturated fatty acids in the control group Also for oleic acid the difference between mean percentages was greater for cholesterol esters (14 2 and 18 7 respectively for the vegetarian and control group) and for triglycerides (35 1 and 39 4) than for phospholipids (10 8 and 12 1) For palmitoleic acid the values were small but all differences statistically significant. For stearic acid the mean values for the cholesterol esters and the triglycerides differed significantly while for palmitic acid only the difference in the triglyceride fraction was statistically significant

Linolenic acid usually appeared only in trace amounts In the triglyceride fraction of the vegetarians however the mean value was 1 2 and significantly different from that of the control group 0 7%

Other significant differences b tween the two

Table III *Triglycerides Fatty acid composition and total fatty acids*

Fatty acids,	Vegetarians *Mean*	*S D*	*Range*	Control group *Mean*	*S D*	*Range*	Result of statistical analysis
Myristic	2 3	1 4	0 6– 6 8	2.5	1 1	1 8 6 4	$p\ \ 0\ 1$
Palmitic	22.8	5 1	10 2–33 3	[illegible]6 1	3 7	21 2–35 3	$p\ \ 0\ 05$
Palmitoleic	4 5	1 5	2 2– 8 7	5 3	1 0	4 2– 6 4	$p < 0\ 05$
Stearic	4 [illegible]	1 3	1 3– 6 9	5 8	1 2	4 3– 7 8	$p < 0\ 001$
Oleic	35 1	4 7	[illegible]5 6–44 6	39 4	5 3	6 6–46 2	$p < 0\ 01$
Linoleic	[illegible]2 3	6 8	11 0–36 4	12 8	2 9	7 9–19 [illegible]	$p < 0\ 001$
Linolenic	1 2	0 4	0– 2	0 7	0 3	0 3– 1 5	$p < 0\ 001$
Arachidonic	1 1	0 3	0 3– 1 6	0 9	0 4	0 4– [illegible]0	$p\ \ 0\ 1$
Eicosapentaenoic?	0 9	1 1	0– 4 4	1 1	0 8	0– 3 0	$p > 0\ 1$
Total fatty acids (mg/100 ml)	65 5	3 [illegible]	33 2–156 5	83 1	35 6	[illegible]6 0–165 [illegible]	$0\ 1 > p > 0\ 05$

Table IV *Phospholipids Fatty acid composition and total fatty acids*

Fatty acids	Vegetarians			Control group			Result of statistical analysis
	Mean	S.D	Range	Mean	S.D	Range	
Palmitic	28.0	3.4	23.1–36.6	27.9	2.1	25.6–34.4	$p>0.1$
Palmitoleic	1.3	0.4	0.4– 2.0	1.5	0.2	1.1– 2.0	p 0.05
Stearic	13.3	1.6	9.9–17.3	13.9	1.1	12.1–16.4	$p>0.1$
Oleic	10.8	1.6	7.4–13.5	1?.1	1.3	10.8–15.0	$p<0.01$
Linoleic	27.0	4.5	17.1–35.4	22.5	2.8	18.0 27.6	$p<0.01$
Eicosatrienoic	1.9	0.8	1.0– 3.5	2.0	0.4	1.1– 2.8	$p>0.1$
Arachidonic	6.3	2.7	?.9–11.0	6.6	1.2	4.8– 9.1	$p>0.1$
Behenic	1.2	0.4	0.6– 1.9	1.7	0.4	0.8– 2.7	$p<0.01$
Eicosapentaenoic?	1.8	1.3	0.5 6.0	2.3	1.5	0.7– 6.1	$p>0.1$
Docosapentaenoic?	1.5	0.5	1.0– 2.8	1.5	0.6	0– 2.8	$p>0.1$
Docosahexaenoic	3.3	2.2	0.7– 8.5	3.7	1.3	2.0 6.2	$p>0.1$
Total fatty acids (mg/100 ml)	84.9	21.4	42.0–1?8.0	100.5	16.5	72.4–142.3	$p<0.01$

Table V *Concentration of fatty acids in cholesterol esters triglycerides and phospholipids*

Fatty acids (mean mg/100 ml)	Cholesterol esters		Triglycerides		Phospholipids	
	Vegetarians	Control group	Vegetarians	Control group	Vegetarians	Control group
Palmitic	7.3	10.0	16.0	23.2	25.9	30.5
Palmitolei	1.8	?.1	3.2	4.7	1.2	1.6
Stearic	1.0	1.8	3.0	5.1	12.3	15.2
Olei	10.5	17.5	24.7	35.0	10.0	1?.2
Linoleic	45.6	49.7	15.7	11.4	25.0	24.6
rachidonic	3.1	4.0	0.8	0.8	5.8	7..

groups in fatty acid percentages were not observed

The dietary survey performed previously in the vegetarian group disclosed a diet relatively rich in polyunsaturated fatty acids but also that great individual variations occurred (12) The individual values for the intake of polyunsaturated fatty acids (in per cent of caloric intake) and for the percentage of linoleic acid in the lipid fractions of serum have been used for calculations of coefficients of correlation From these calculations it appeared that there was a positive correlation between these parameters in all fractions The coefficients of correlation were significant for the cholesterol ester and triglyceride fractions (0.6284 $p<0.01$ and 0.5640 $p<0.01$ respectively) and close to significant for the phospholipids (0.3998 $0.1>p>0.05$)

The absolute total fatty acid concentrations of cholesterol esters (Table II) triglycerides (Table III) and phospholipids (Table IV) appeared to be lower in the vegetarian group than in the control material The differences were statistically significant for the cholesterol esters and phospholipids but only close to significant for triglycerides

In Table V the results of a calculation of concentrations expressed in mg/100 ml for some of the main fatty acids in the cholesterol esters the triglycerides and the phospholipids are re

Table VI *Fatty acid composition of lipid fractions in healthy individuals Range of means from different publications*

Fatty acids	Cholesterol esters	Triglycerides	Phospholipids
Palmitic	10.3 15.6	24.9–32.0	27.7–37.0
Palmitoleic	2.7– 8.4	3.1– 7.8	1.1– 3.6
Stearic	0.8– 3.5	4.0– 6.8	11.9–15.3
Oleic	17.8–24.0	36.0–45.0	11.3–17.8
Linoleic	44.9–57.6	9.5–17.9	18.2–24.3
Arachidonic	3.9– 8.4	0.1– 2.4	6.2–14.2

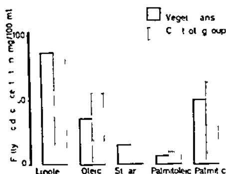


Fig 1 Sums of fatty acid concentration in cholesterol esters triglycerides and phospholipids

corded The table shows that the linoleic acid concentration of the cholesterol esters in serum is greater for the control group than for the vegetarian group In the phospholipids the linoleic acid concentration is the same in the two groups while in the triglycerides it appears higher in the vegetarian group The sums of linoleic acid concentrations from all three fractions are illustrated in Fig 1 and are practically identical in the two groups Thus the large difference in percentage composition of linoleic acid of the two groups is not evident when concentrations are considered

On the other hand the differences in some monounsaturated and saturated fatty acids particularly oleic stearic and palmitic acid are greater when concentrations rather than percentage composition are considered and appear quantitatively as the main differences between the two groups (Fig 1)

COMMENTS

The results regarding absolute values for the fatty acid moiety of cholesterol esters and phospholipids confirm the previous findings of lower values of these lipids in vegetarians (11 12)

The percentage composition of fatty acids in blood in the control group healthy men aged 40 to 70 years may be regarded as normal" values for the population group they represent Quantitatively the most important fatty acids were linoleic oleic and palmitic acid to a minor degree also palmitoleic stearic and arachidonic acid In Table VI the range of mean values for these acids obtained in healthy adults and taken from publications from USA West Germany and Sweden are recorded (3 7 15 19 20 21 23) The table reveals great variations of values a fact which may be due to differences in dietary habits or in methods However in all studies a characteristic pattern in the distribution of fatty acids is apparent in the three lipid fractions This pattern is observed also in the present study and all mean values of the control material fall within the range of values from the publications cited

The vegetarians reveal a percentage composition which for many fatty acids falls outside the range of normal values given in Table VI This is particularly true of linoleic and oleic acid for which the values in all lipid fractions fall outside the range of normal values and are significantly different from the control material of the study In some fractions also palmitic palmitoleic and stearic acid fall outside the range of normal values and/or differ significantly from the control material

The effect of polyunsaturated fatty acids in the diet on the fatty acid composition of serum or plasma lipids has been the subject of a large number of experimental studies These studies nearly always have considered percentage composition of the fatty acids Experiments with widely different levels of linoleic acid have been performed in animals and in man (1 6 9 10 14 16 23) A pattern of characteristic variations in the serum fatty acids has been found The most consistent findings are a lowering of linoleic acid in serum on an essential fatty acid deficient or low fat diet and an increase when linoleic acid rich diets are fed The changes in linoleic acid percentages in serum are balanced by variations in oleic acid and often in palmitoleic acid also Usually both these monounsaturated fatty acids increase in the lipid fractions when dietary linoleic acid is lowered while a decrease is apparent when the linoleic acid in the diet is increased Changes in palmitic and stearic acid also occur but are less consistent

The results regarding arachidonic acid in the experimental studies are more conflicting In man the percentages of arachidonic acid undergo only small changes when dietary linoleic acid is changed (6 9 10 23)

When compared with the results of the experimental studies cited above the findings in the vegetarian group are in accordance with the high intake of polyunsaturated fatty acids demonstrated in the previous dietary survey of the

same group The significant positive coefficients of correlation between polyunsaturated fatty acids in the diet and the linoleic acid percentages in cholesterol esters and triglycerides of the vegetarians indicate that the high intake of linoleic acid is the main cause for the high values of linoleic acid in these lipid fractions

Studies of the fatty acid composition of serum have been performed in vegetarian populations of low socioeconomic standard in Africa and Asia. In groups consuming a low fat low-cholesterol diet, low percentages of linoleic acid in serum have been demonstrated (18 21) In a study from India it was demonstrated that vegetarians and non vegetarians on similar intakes of polyunsaturated fatty acids also had similar values for the percentages of dienoic acids in the blood An increase in the dienoic acid values could be induced in the vegetarians by supplementation of the diet with sesame oil (16) Evidently therefore the intake of polyunsaturated fatty acids is the major factor regulating the linoleic acid in the blood of the vegetarian groups while cholesterol intake or other possible particularities of the vegetarian diet are of minor importance

The present study gives an indirect confirmation of the results obtained in the previous dietary survey performed in the same subjects Since the two studies were performed at an interval of 6 to 12 months the significant relationships between dietary and serum linoleic acid also demonstrate a consistency in the dietary habits of the group

Nearly all the experimental studies of the effect of variations in dietary intake of linoleic acid on the serum linoleic acid have considered percentage composition of the fatty acids in serum The comparisons between the two groups in Tables II III and IV have therefore used this parameter If absolute values are considered (Fig 1) the concentrations of linoleic acid appear similar in the two groups and the differences in concentrations appear as an excess of some saturated and monounsaturated fatty acids in the control group These observations do not affect the validity of the conclusions drawn above It is very well documented that the effect of a substitution of polyunsaturated for saturated fats in the diet is not only to change the percentage fatty acid composition, but also to lower the serum lipid levels The results in Fig 1 therefore are also in agreement with the dietary survey

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THE FATTY ACID COMPOSITION IN SERUM FOLLOWING MYOCARDIAL INFARCTION

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Abstract Studies of cholesterol ester triglyceride and phospholipid fatty acids have been made on the first and on the fifth day following an acute attack of myocardial infarction in ten men Statistically significant decreases in total fatty acids of these fractions were observed Gas-chromatographic analysis of the fatty acid composition showed a decrease in linoleic acid percentages in all the three fractions balanced by an increase in some saturated and mono-unsaturated fatty acids

It is concluded that dietary factors with a low fat diet due to nausea and anorexia, may play a role in the variations in serum lipid concentrations in this period of the myocardial infarction However hormonal factors affecting lipid metabolism may also be of importance Studies are in progress to elucidate further the relative importance of the dietary factors

One additional conclusion which may be drawn from the variations in fatty acid composition observed is that studies of fatty acid pattern in acute myocardial infarction are not a reliable guide to the preinfarction dietary habits of the patients

A decrease in serum lipid values has been observed in the course of myocardial infarction (23) Studies of total cholesterol have shown that the values are at their lowest 2 to 17 days on the average eight days after the onset of the infarction with a subsequent increase thereafter (16) The exact mechanism responsible for these variations is unknown but they have been attributed to the changes in endocrine homeostasis caused by the acute stress (13) Several of the hormones involved in the alarm reaction are known to affect lipid metabolism A decrease of cholesterol (3) as well as of triglycerides (5) has been observed after the administration of ACTH

However in many of the patients with myocardial infarction the severe condition is complicated by nausea and anorexia with impairment in the intake of food It therefore seems appropriate to consider also a low fat diet as a factor possibly contributing to the changes in lipid metabolism This report describes the effects of myocardial infarction on the fatty acid composition of cholesterol esters triglycerides and phospholipids of serum Since studies of the fatty acid pattern have been proposed as an objective guide to dietary fatty acid intake (7 9 14) it was hoped that the study would yield information elucidating the importance of dietary factors in the mechanisms leading to a fall in serum lipid values in myocardial infarction

MATERIAL AND METHODS

The study comprised ten men aged 45 to 75 years suffering from myocardial infarction The diagnosis was based on usual criteria including the characteristic symptoms progressive changes in the electrocardiogram, and elevated values of glutamic oxalacetic transaminase and creatine phosphokinase Only patients from whom blood specimens for lipid analyses could be obtained within 24 hours following the initial attack of pain were included in the study Blood was drawn after a 12 hour fast on the first and on the fifth day after admission to the hospital

The separation of cholesterol esters triglycerides and phospholipids was performed by column chromatography and the purity of fractions was tested by thin layer chromatography The lipid fractions were hydrolysed and methylated according to the method of Stoffel et al (2) The determination of the fatty acid composition of the methyl esters was made by gas-chromatography The procedure as well as the methods used for column and thin layer chromatography have been described elsewhere (1)

Aliquots of the lipid fractions obtained by column chromatography were pipetted off for analyses of total fatty acids The hydrolysis of these aliquots was performed according to the method of Pikaar and Fernandes (19) and the titration of fatty acids according to a modification of Dole s method (4)

The statistical analyses of the results were done by Student s *t* test for paired data

Table I. *Fatty acid composition and total fatty acids of serum lipid fractions on the first and fifth day of myocardial infarction*
Mean, S.D. and result of statistical analysis

Fatty acids	Day	Cholesterol esters		Triglycerides		Phospholipids	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
Palmitic	1	13.4	1.5	30.7	4.1	38.0	5.3
	5	14.3	1.7	32.2	3.0	41.2	6.9
						$p<0.05$	
Palmitoleic	1	4.4	2.0	6.4	1.9	1.5	0.7
	5	4.5	1.3	5.7	1.5	1.8	0.8
Stearic	1	1.2	0.5	3.4	1.3	13.2	1.4
	5	1.3	0.5	4..	1.2	12.7	2.2
Oleic	1	18.8	3.6	38.8	3.0	1.0	2.0
	5	20.5	4.4	39.1	4.4	12.1	2.6
		$p<0.05$					
Linoleic	1	54.4	4.6	12.8	4.1	19.8	1.9
	5	51.2	6.1	10.7	3.0	17.4	2.8
		$p<0.05$		$p<0.05$		$p<0.05$	
Arachidonic	1	2.4	1.5	0.4	0.4	3.2	1.1
	5	2.2	1.3	0.3	0.3	2.6	1.4
Total fatty acids	1	76.1	20.6	125.2	49.3	104.8	35.9
mg/100 ml	5	61.7	16.9	80.0	35.4	84.8	.6.1
		$p<0.05$		$p<0.05$		$p<0.05$	

RESULTS

The results are recorded in Table I, giving the mean values and the standard deviations (S.D.) of total fatty acids in mg/100 ml and fatty acid composition in per cent of total fatty acids in cholesterol esters, triglycerides and phospholipids in the sera from the first and fifth day. Several fatty acids were present in small amounts, but the table records the quantitatively most important fatty acids only.

The total fatty acids of the three lipid fractions appear significantly lower on the fifth than on the first day, confirming the decrease in serum lipids observed by other authors during this phase of the myocardial infarction.

From the table it is apparent that changes in the fatty acid composition also occur. In all fractions the percentage of linoleic acid is significantly lower on the fifth than on the first day: for cholesterol esters 51.2 as against 54.4, for triglycerides 10.7 against 12.8, and for phospholipids 17.4 against 19.8%, respectively.

The decrease in linoleic acid is balanced by changes in some saturated and monounsaturated fatty acids. Thus the percentage of oleic acid in the cholesterol ester fraction is significantly higher on the fifth than on the first day, 20.5 and 18.8%, respectively. In the phospholipid fraction palmitic acid is significantly higher on the fifth day, 41.2 against 38.0% on the first day. In the triglycerides the percentages of palmitic and stearic acid are higher on the fifth day, but the differences are not statistically significant.

The decrease in linoleic acid values was observed in all patients in the triglyceride fraction and in nine of the ten patients in the cholesterol and phospholipid fraction.

DISCUSSION

The decrease in linoleic acid percentages occurring in the lipid fractions during the first days of the myocardial infarction indicate a low intake of linoleic acid in this period. A low fat diet is known to effect a decrease in linoleic acid percentages and a relative rise in some of the saturated and monounsaturated fatty acids (6, 15, 20, 21). The patients reported here were given a hospital diet relatively rich in fat. The nausea and anorexia with impaired intake of food, including fat, during the first days of myocardial

infarction may therefore possibly be responsible for the lowering of linoleic acid values in this study A low fat diet may very well contribute to the fall in cholesterol values However for triglycerides this explanation would not be entirely satisfactory It is known from experimental studies that triglycerides actually increase during short periods of low fat diet (1 8 10) In our patients we found a decrease also in the triglyceride fraction The present study therefore has not excluded hormonal factors as responsible for some of the variations in serum lipids but demonstrates changes in fatty acid composition indicating that also dietary factors may be involved Combined effects of dietary and hormonal factors are possible and studies designed further to elucidate the relative importance of the diet are in progress

Studies of the fatty acid composition of serum have been used as a guide to the quality of fat intake and may be of great interest in the study of the dietary habits of patients with coronary disease However no conclusion regarding preinfarction values can be drawn from any study of variations of the fatty acid pattern during the acute phase of myocardial infarction

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EFFECTS OF REPEATED WORK TESTS AND ADRENERGIC BETA BLOCKADE ON ELECTROCARDIOGRAPHIC ST AND T CHANGES

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Abstract The effect of a number of repeated work tests on different electrocardiographic ST and T changes has been studied and the effect compared with that after an adrenergic beta receptor blockade

In subjects with different ECG changes but without any apparent organic heart disease there was a normalization of the ST and T changes during the period of repeated work tests. The normalizing effect was almost identical to that after adrenergic blockade Different patterns of ST and especially T depressions on the ECG at rest, in the standing position and during and after work are described

In patients with organic heart diseases the repeated work tests generally had no effect on the ECG patterns typical of coronary insufficiency and myocarditis In one patient with signs of a vasoregulatory disturbance there seemed to be ST and T changes of different origin, probably both functional and organic The effect of the adrenergic blockade was almost identical to that of the repeated work tests

The study gives support to the results of a previous investigation in which the possibility of using an adrenergic blockade for differentiating between ECG changes of functional and organic origin was pointed out

The normalization effect of 4–6 work tests over a period of 4–6 days on functional ST and T changes on the ECG is supposed to be due to a physical and psychic adaptation to the work tests and to the examination situation

There are several ECG studies on the occurrence of marked ST depressions and especially T wave inversions in apparently healthy subjects (4 10 16 17 21 25) These ECG changes of the ST T interval are sometimes similar to those occurring in organic heart diseases and often give rise to difficulties in differential diagnosis (14 15 21 28 32)

It is a well known fact that adrenergic stimulation of the heart particularly after the injection of adrenaline produces changes of the ST T interval in humans (5 22 29) The resemblance of these ST and T changes to those mentioned above has led to a characterization of the latter as functional or sympathicotonic (14 23)

A higher frequency of electrocardiographic ST and T changes with no other signs of heart disease have been reported in certain groups of diseases as compared with healthy subjects This is true of patients with neurocirculatory asthenia (11 32) vasoregulatory asthenia (14) and various psychiatric diseases (20 27 28 31) A predominant symptom in these patients with functional ST and T depressions on the ECG is generally anxiety (18 19 25 27 30 32) The ECG changes often accompany changes in the course of the disease or the patient s mental state and may disappear when the patient recovers (18 19 24 30)

Functional ST and T depressions are decreased or disappear after a period of physical training according to Holmgren et al (14) They also disappear after a pharmacological blockade of the adrenergic stimulation of the heart (1 7) In a previous study the possibility of using an adrenergic beta receptor blocking agent to differentiate between ECG changes of functional and organic origin was pointed out (7) In this investigation it was noticed that functional ST and T changes of the ECG might have different patterns and that they may occur together with ECG changes of organic origin.

The purpose of the present study was to analyze the effect of repeated work tests on different patterns of ST and T depressions recorded at rest, in the standing position and in connection with a work test The study includes patients with and without heart disease and the effect of the repeated work tests on the ECG changes has been compared to that after an adrenergic beta blockade

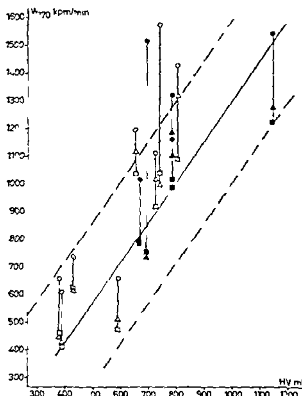


Fig 1 Relationships between physical working capacity at pulse 170 (W 170) and heart volume (*HV*) in eight subjects with functional ECG changes (*unfilled symbols*) and five patients with organic heart diseases (*filled symbols*) □ 1st work test ○ 2nd work test performed during adrenergic beta blockade △ the final work test after 4–6 repeated tests Regression line ±2 S.D from 58 healthy subjects of various age sex and degree of physical training (1)

MATERIAL

One part of the material (group I) consisted of subjects with ST and T changes on the ECG but no other signs consistent with any organic heart disease From a material of almost one hundred subjects fulfilling these criteria and previously examined with ECGs before and during an adrenergic beta blockade eight co-operative subjects four women and four men aged 14–36 years were selected They were characterized by having different ST and T changes on the ECG The auscultation of their hearts was normal, as well as their chest X ray There were no criteria for suspecting any organic heart disease anamnestically or clinically All subjects except one had an ordinary physical working capacity at pulse 170 (W 170) in relation to heart volume (HV) and the total amount of hemoglobin (THb) One woman had a somewhat low W 170 in relation to the circulatory dimensions HV and THb and she may be regarded as a less advanced case of vasoregulatory asthenia (13) (Fig. 1) All subjects in this group were previously examined by ECG and in six of them the ST and T changes on the ECG recorded at the beginning of the study had existed for more than one year *Three subjects in this group were examined durin a stay* at the hospital caused by a psychic disease The rest were healthy and fully employed Three of the latter group were inclined to emotional stress

The other part of the material (group II) included patients with ST and T changes on the ECG and other signs of an organic heart disease This group consisted of five men aged 19–59 years who had been treated for their heart diseases at a medical clinic. Two of them (A. N and T H) had for more than two years angina of effort (criteria stated by WHO (33)) They had precordial pains during work which were noticed at the repeated work tests The ECG recordings during work revealed ST and T depressions typical of coronary insufficiency (cf 7) One patient (B L.) was examined during the course of an acute myocarditis He had signs of a streptococcic infection with an elevated antistreptolysin titer of 1600 units *maximally His ECG changes at rest showed a typical* development and disappeared completely within one month The remaining patients in this group (L. E. J and L. S) had electrocardiographic signs of sequelae after a myocarditis One of them was treated at a medical clinic nine years ago for an acute myocarditis The other one has been suffering from palpitations breathlessness and a decreased physical working capacity after a prolonged common cold accompanied by a high temperature The clinical diagnosis was sequela after a myocarditis The relationships between the physical working capacity at pulse 170 and the heart volume of the patients in this group are given in Fig 1

METHODS

The ECGs were recorded for each patient at rest in the supine position, in the standing position and during and following a standardized work test Standard leads I II, III and precordial leads CR_1 CP, CR CR and CR were recorded by a direct writing ink jet recorder (Mingograph 42 or 81 Elema Schonander Stockholm) During the exercise on a bicycle ergometer the reference electrode was placed on the patient s forehead The methods used in the study were identical with those in the previous study (8)

The patients performed 4–6 standardized work tests over a period of 4–6 days i e one test a day The second work test was performed by each subject durin an *adrenergic beta receptor blockade (after peroral administra*tion of propranolol) according to a method previously reported (7) Two of the patients (B L and T H) performed more tests, ten and eight respectively in the corresponding number of days The patients were asked to work until they were unable to continue This usually occurred when the pulse rate reached 170–180 beats/min. The two patients with coronary insufficiency worked until they had precordial pains

The physical working capacity was calculated at pulse 170 beats/min by inter or extrapolation assuming a linear relationship between pulse rate and work load (W 170)

The work tests were usually carried out at the same time of day for each subject to avoid the possible influence of a diurnal variation They were performed at

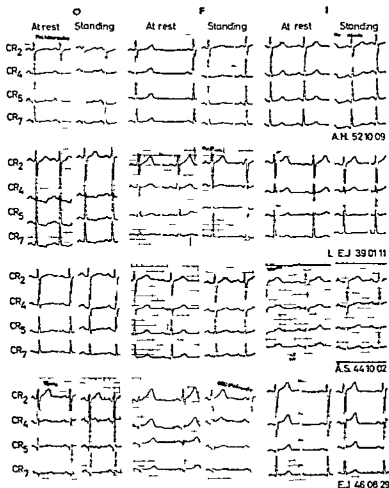


Fig 2 Precordial ECG leads at rest and in the standing position in four subjects with different functional ST and T chan es O ECGs from the first test *F* ECGs from the final test after a period of repeated work tests *I* ECGs during adrenergic beta blockade (test no II)

least three hours after a meal to avoid postprandial influence The patients were asked not to smoke within one hour of the work tests

RESULTS

The study has shown that electrocardiographic ST and T changes of different patterns recorded at rest in the standing position and in connection with a work test in subjects without apparent signs of organic heart disease were more or less completely normalized after a number of repeated work tests The effect of these tests on the ECGs was similar to that found after an adrenergic beta receptor blockade

There was a different response to the repeated work tests in patients with organic heart diseases The ECG patterns typical of coronary insufficiency myocarditis and sequela after myocarditis were generally not affected nor were they affected by the blockade

ECG at Rest

Group I

The ST and T depressions on the ECG at rest had different patterns One consisted of slight ST and T depressions in the left precordial leads as illustrated in Fig 2 (case A H) Sometimes there were only ST depressions and a normal positive T wave A second pattern of ECG changes of the ST and T interval in this group consisted of localized T wave depressions in the apex area, i e in lead CR_4 A slight change of this type is seen in Fig 2 (case A S) while it is more prominent in another subject (Fig 5) A third ECG pattern may be characterized by marked ST and T depressions in the left precordial leads A fourth type of ECG change included T wave inversions associated with an elevated ST interval Slight T wave changes of this pattern were recorded in one patient E J (Fig 2) They are better illustrated in a previous work (7)

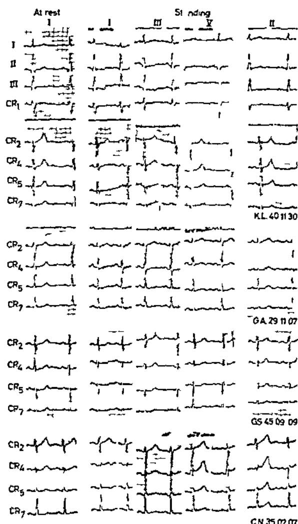


Fig 3 Precordial and extremity ECG leads at rest and in the standing position in four subjects with functional ST and T changes recorded during a period of repeated work tests The Roman figures indicate in which test during the period the ECGs were recorded II means second test during an adrenergic beta blockade

Group II

In two patients there was a normal ECG at rest One had slight ST depressions and another T wave inversions that were not or only to a slight degree affected by the repeated work tests as well as by the beta blockade (Fig 4) In the fifth patient there was a normalization of the marked ST and T changes at the final examination The effect of the beta blockade was similar to that of repeated work tests (Fig 4)

ECG During the Orthostatic Test

Group I

The different patterns of ST and T changes on the ECG at rest could also be recognized during an orthostatic test The ECG changes were generally accentuated as is clear from Fig 2 In many cases the ST and T changes appeared at first in the standing position (Fig 3)

During the period of repeated work tests there was a successive normalization of the ECGs which is most obvious in the cases K L and C N (Fig 3) The ECG changes during the orthostatic test seemed to be sensitive to slight emotional stress Thus there was an interruption of the successive normalization of the ECG changes on the day when one of the subjects gave a talk to his class in school and in another patient on the day when he was to be demonstrated to medical students at a lecture

Group II

In one patient with slightly accentuated T wave inversions in the standing position and another who had slight T wave depressions there was no influence of the repeated work tests on these additional changes A third patient with marked ST T depressions had a normal ECG at the final examination (Fig 4)

ECG in Connection with Work

Group I

During exercise at increasing work loads the ST and T changes recorded at rest usually decreased In a few cases marked ST and T depressions were recorded during exercise at a low work load while they decreased when the work intensity was higher (Fig 5) This was true mainly of subjects with marked ST and T changes during the orthostatic test ECG changes when recorded at rest or in the standing position were usually recorded also 4 min after the work It sometimes happened that slight ST and T changes at rest before the work disappeared 10 min after it

Group II

The abnormal ST and T depressions recorded during work in the patients with coronary insufficiency and sequela after myocarditis were not or at least only insignificantly influenced by the period of repeated work tests Nor was there

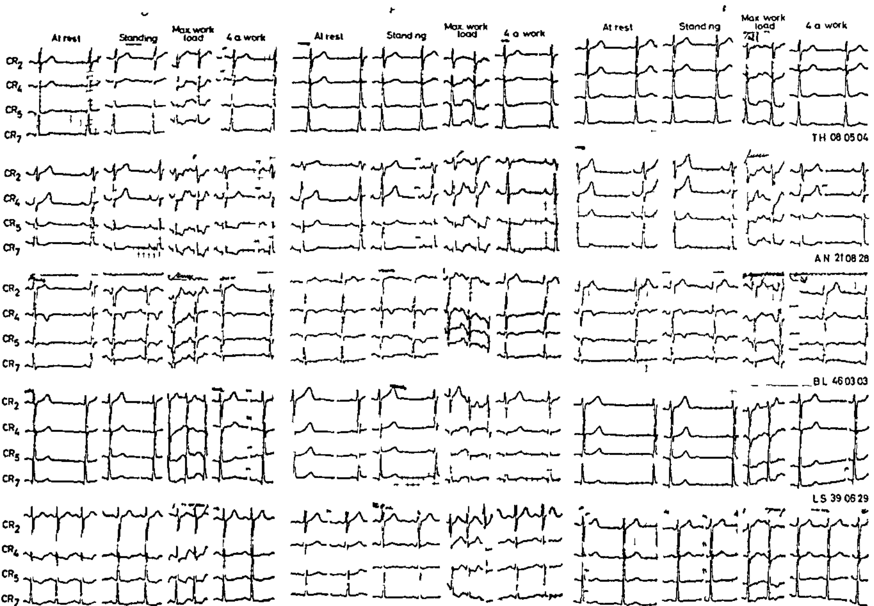


Fig 4 P e d lECG l d at e t i th st d g po t d 4 m n ft r w k n from tl f l t t ft r p d f pe t d w k te t I ECG l l gad e e g c
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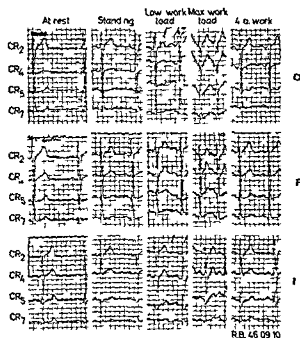


Fig 5 Precordial ECG leads at rest in the standing position during exercise at low and maximal work load and 4 min after work in a subject with functional ST and T changes O ECGs from the first test F ECGs from the final test after a period of repeated tests I ECGs during adrenergic beta blockade (test no II)

any significant effect on these abnormal ST and T changes during the beta blockade while there were some effects on the T wave depressions 4 min after work This observation that T wave changes in patients with coronary insufficiency are less pronounced after work in connection with an adrenergic beta blockade has been reported earlier (7)

W_{170}

There were generally small changes in W_{170} after the period of repeated work tests in both groups During the blockade there were greater increases in W_{170} and they were generally of a magnitude previously reported as occurring in healthy subjects with an ordinary W_{170} (Fig 1) In one patient (L E J) with marked ECG changes the increase in W_{170} during the blockade was not very different from that in cases with vasoregulatory asthenia (9)

DISCUSSION

The way in which the period of repeated work tests influenced the electrocardiographic ST and T changes is not quite clear There was obviously a different response to the period of repeated work tests in the two groups In patients with vasoregulatory asthenia it has earlier been reported that ST and T depressions of functional origin disappeared or decreased after a period of physical training (14) There is no report about the effect of repeated work tests on ECG changes of organic origin but there is no reason to believe that they would be affected A different response to an adrenergic beta receptor blockade was also noted in the two groups In group I the different ST and T depressions disappeared during the blockade while the corresponding ECG changes in group II were generally unaffected The effect of the blockade was almost identical to that after a short period of repeated work tests In a previous animal study (3) it was shown that ST and especially T wave inversions occurring on the ECG after experimentally induced myocardial damage were not affected by an adrenergic beta receptor blockade The chance that the ECG normalization in group I would coincide with an ECG normalization in the course of an acute myocarditis seems to be minimal There were no clinical or laboratory signs of an infection in any subject except for case B L in group II Other considerations opposing such an assumption are the fact that most subjects have had their ECG changes for more than one year the rapid regress of the ECG change during the course of 4–6 days and the fact that the ECG changes in the only subject with signs of current infection were not influenced Thus it seems very probable that the different ST and T depressions in the ECG in group I are of a functional origin and that the ECG changes in group II are largely of another origin most likely an organic one

The normalization of the functional ECG changes during the repeated work tests is probably due to a physical and psychic adaptation to the work tests and to the examination situation by the different individuals Magendantz et al (19) have pointed out that functional ECG changes at rest may disappear when the recording is repeated after reassurance and rest Ljung (17) has called attention to the fact that certain patients are frightened by the number of electrodes coupled to extremities and thorax during an ECG recording However no reassurance was given to the subjects but there was an im

pression that the patients were less tense as the period of repeated work tests proceeded Holmgren et al. (14) have reported that the normalization of functional ECG changes of the ST and T interval accompanied an increase in the low physical working capacity that characterized patients with vasoregulatory asthenia (VA) In previous studies (1 7 8) it has been suggested that one patho physiological mechanism in the VA syndrome is a relative increase in sympathetic tone It is commonly thought that physical training may cause the autonomic balance to deviate in the vagotonic direction It is feasible that this is one part of the mechanism behind the normalizing effect of the repeated work tests A similar autonomic deviation in the vagotonic direction was obtained in a pharmacological way by the adrenergic blockade The resemblance between the ECGs after the blockade and after the series of work tests is obvious

The most common functional ST and T changes on the ECG at rest were localized in the left precordial leads They may consist of slight ST depression with a normal T wave slight ST and T depressions and marked ST depressions with T wave inversions Probably they express a varying degree of functional disturbance T wave changes characterized by a negative first component and a positive second component similar to those induced by adrenaline (5 29) were commonly seen in this study

Isolated T wave depressions are usually not interpreted as functional Littman (16) has reported that T wave inversions in healthy young men are most predominant around the apex lead It must however be pointed out that similar isolated T wave depressions have been observed in patients with myocarditis In these cases the T wave change was not affected by a beta adrenergic blockade The different reaction to an adrenergic blockade may indicate that there may be a difference in the genesis of the isolated T wave changes An unusual pattern of functional ECG changes is T wave inversions associated with elevation of the ST interval This has been observed previously by other authors (4 10)

Functional ECG changes usually appeared or were accentuated during the orthostatic test. The electrocardiographic changes induced by this test are usually believed to be caused by vegetative or autonomic disturbances primarily an increased sympathetic tone (6 14 17 23) In some patients at the final test there was not a complete normalization of the functional ST and T interval in the standing position This was true of subjects with marked functional changes as well as of some patients with organic heart diseases An insufficient number of work tests might have been performed by these subjects However Holmgren et al (14) made a similar observation in their study of patients with vasoregulatory asthenia Functional ST and T changes on the ECG at rest disappeared after physical training while there was no complete normalization of these changes during the orthostatic test. Another explanation may be true of the patients with organic heart diseases A diseased heart seems to be more sensitive to sympathetic stimulation judging from experimental ECG animal studies (2 26)

The functional ST and T depressions recorded on the ECG at rest and during the orthostatic test were usually reduced as the intensity of the work tests increased They returned afterwards especially 4 min after the test. In some cases marked ST and T changes were observed during work of a low intensity Stevenson et al (30) have reported that ST and T changes recorded after work in some patients with anxiety were less pronounced when the mental condition of the patients was improved

Another ECG pattern of functional origin during work was found in patients with vasoregulatory asthenia (14) The ST and T depressions on the ECG at rest increased during the orthostatic test as well as during muscular work The ECG changes were just as marked at low as at high work loads

The ECG changes of organic origin were generally not influenced by the repeated work tests as could be expected This was true of one patient with acute myocarditis and three cases with coronary insufficiency or sequela after myocarditis These ST and T changes were not influenced to a great extent by the adrenergic beta blockade This is in accordance with previous reports (3 7)

The marked ST and T depressions recorded at rest and in the standing position in one patient with sequela after a myocarditis disappeared after the repeated work tests while the ST and T depressions during heavy work were unaffected

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INVESTIGATION OF PATIENTS WITH MILD THROMBASTHENIA — A HAEMORRHAGIC DISORDER WITH PROLONGED BLEEDING TIME PROBABLY DUE TO A PRIMARY PLATELET DEFECT

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Abstract Fifteen families with a haemorrhagic disorder are reported The patients had a bleeding tendency characterized by easy bruisability nose bleeding menorrhagia and bleeding after tooth extractions accidents or surgery Laboratory investigation showed prolonged Ivy bleeding time and usually decreased platelet adhesiveness as measured by Hellem s whole blood method Salzman s method and occasionally Hellem s plasma ADP method Factor VIII and other coagulation factors were normal The prothrombin consumption test was mostly normal and there was no pathologic fibrinolysis The inheritance was probably dominant but the penetrance weak The disease was probably caused by a primary platelet defect especially since a similar syndrome was found among relatives of a family with severe thrombasthenia of Glanzmann s type We call the condition mild thrombasthenia

Nose bleeding bleeding after tooth extraction ready bruisability and menorrhagia often suggest a haemorrhagic diathesis Investigation of patients with such symptoms often reveals a prolonged Ivy bleeding time but a normal platelet count The coagulation factors are normal and there are no signs of pathologic fibrinolysis but the platelet adhesiveness as measured by Hellem s or Salzman s methods is decreased Clinical examination shows no systemic disease Such patients are probably common and patients with a bleeding tendency and prolonged Ivy bleeding time as the only signs of disease have been reported by Jacobson (15) and Larrieu (16) As the condition and laboratory findings are usually mild the condition is often ignored

This study reports the clinical and laboratory findings in 28 patients belonging to 15 families with mild bleeding symptoms and prolonged Ivy bleeding time but normal factor VIII and platelet count

MATERIAL AND METHODS

Human fraction I-0

Human fraction I-0 containing AHF (factor VIII) was prepared at the Chemistry Department II Karolinska Institutet Stockholm, by the glycine method of Blomback and Blomback (3) One dose of fraction I-0 is prepared from 1400 to 1600 ml of fresh normal plasma and contains about 3 g of protein Usually one half of one dose of fraction I-0 was given on each occasion Half a dose of fraction I-0 dissolved in 100 ml isotonic saline has an AHF activity 5 to 8 times that of 100 ml fresh normal plasma.

Collection of blood was performed in the way described previously (6)

Coagulation tests All methods used for preparing the blood samples and for determining the coagulation factors have been described elsewhere (19) The factor VIII activity of plasma was assessed by its normalizing effect on the recalcification time of haemophilia A plasma (18 19 20) and the amount of AHF (factor VIII) present was expressed as a percentage of that found for a normal standard consisting of pooled plasma from 10 individuals.

The *bleeding time* was determined according to the methods of Duke and Ivy (7 21) The Ivy bleeding time was measured by inflating a blood pressure cuff to 40 mm Hg and making three transverse standardized incisions on the volar aspect of the forearm with a sharp surgical blade (Gillette Surgical Blade E) In 35 normal individuals the mean was 9 5 min (range 5 to 15 5 min)

The *fibrinolytic system* was studied in the way described by Nilsson and Olow (22, 23)

Platelet counts were made by Hellem s modification (11) of Nygaard s method (25) Control counts were also made by the method of Bjorkman ( )

Platelet suspensions were prepared and tested for platelet factors 1 3 and 4 as described previously (24)

Platelet rich plasma was prepared from citrated blood (one part 3 8% sodium citrate and nine parts blood) drawn by the silicon technique and centrifuging at 185 g for ten min immediately after collection.

Platelet-deficient plasma was prepared by centrifuging platelet rich citrated plasma at 16 000 g for 30 min in plastic tubes at 4 C and then carefully pipetting off the

Table I *Coagulation analysis*

Family	Coord no	Initials	Sex	Born	Bleeding time: Duke (min)	Bleeding time: Ivy mean (min)	Ivy bleeding time Frequency distribution: <15	15–25	>25 min	Platelet count (mm³)
1	II 1	K L	♀	1915	2–5	>30	0	3	8	2[illegible]6 000
	III 2	G L	♀	1944	2–4	18	0	3	0	2[illegible]1 000
	III 3	G L	♂	1946	2–3	17	1	1	0	176 000
	III 4	E L	♀	1955	2–3	18	1	1	1	269 000
	III 1	B L	♂	1943	2–3	12	1	0	0	190 000
	IV 1	J A	♂	1965	3	13	1	0	0	
2	III 5	L O	♂	1957	3–6	>30	0	4	3	26[illegible] 000
	III 2	J O	♂	1946		>30	0	1	2	163 000
	II 3	I O	♀	1922	2–4	18	1	6	0	230 000
	II 1	K O	o	1924	2	12	1	0	0	187 000
	III 1	I O	♀	1944	1–2	10	3	0	0	210 000
	III 4	A B O	♀	1949	1–3	18	1	1	0	235 000
	III 3	B O	♂	1958	1–2	15	1	2	0	210 000
3	IV 5	H M	♀	1933	2–5	22	2	6	5	210 000
	V 2	T M	o	1954	1–5	16	3	2	1	201 000
	V 3	I M M	♀	1956	2–4	20	1	3	1	210 000
	V 4	T M	♂	1959	2–3	16	2	4	0	210 000
	V 1	K M	♀	1953	2–4	22	0	5	1	149 000
	III 3	E W	♂	1906		10	1	0	0	180 000
	III 9	C W	♀	1900		7	1	0	0	240 000
	IV 4	K E M	o	1926		8	1	0	0	227 000
4	III 2	J W	♂	1952	2–10	>30	0	1	9	276 000
	III 1	H W	♀	1949		25	0	1	0	
	II 6	U W	♀	1929	1–5	25	1	2	3	216 000
	III 3	E W	♀	1954		9	1	0	0	188 000
	I 4	M L	♀	1908		17	0	1	0	243 000
5	III 1	E N	♀	1951	3–5	>30	0	2	4	368 000
	II 2	B N	♀	1927		16	1	2	0	240 000
	II 1	F N	♂	1928		9	1	0	0	259 000
6	III 2	M J	♂	1959	2–9	16	1	1	0	[illegible]04 000
	II 3	S J	♀	1931	2	20	0	2	0	176 000
7	II 2	O S	♂	1962	4–6	22	0	3	1	2[illegible]4 000
	I 1	S S	♂	1936	6–10	22	1	0	1	136 000
	I 2	U S	♀	1935	2–4	18	0	3	0	186 000
	II 1	A S	♂	1960		15	0	1	0	247 000
8	II 3	I B	♀	1912	[illegible]–10	22	0	6	0	2[illegible]4 000
	III 2	Ö B	♂	1944		15	0	1	0	232,000
	III 1	*B B*	♂	*1937*		*11*	*1*	*0*	*0*	[illegible]2 000
9	II 1	A Å	♀	1931	3–4	25	0	2	2	250 000
	I 2	G L	♀	1906		18	0	2	0	270 000
	III 2	B G Å	♂	1949	1–6	14	1	0	0	244 000
	III 3	T Å	♂	1956						301 000
	II 2	J E L	♂	1941	2	15	0	1	0	[illegible]56 000
10	III 2	E B	♀	1923	1–5	25	0	5	4	[illegible]00 000
	IV 1	M Ö	♀	1944		18	0	1	0	19[illegible] 000
	V 1	A Ö	♀	1963		20	0	1	0	209 000
	II 2	E H	♀	1891		29	0	0	1	186 000
11	II 1	D H	♀	1913	3–8	23	0	6	1	[illegible]46 000
	III 2	B O	♀	1938		15	0	1	0	178 000
	IV 1	C O	♂	1962		11	1	0	0	
	III 1	L H	♂	1933		6	1	0	0	201 000
12		A O	♂	1938	4–8	18	1	5	0	241 000
13	II 3	I E	♀	1928	1–3	26	0	3	3	23[illegible] 000
14		E S	♀	1958	5–19	>30	0	0	1	248 000
15	III 2	C H	♀	1949	4–5	>30	0	1	4	234 000
	II 3	M H	♀	1918	2–3	18	0	4	0	194 000
	III 3	E A	♀	1955	2–6	21				[illegible] 000
Normal					1–4	6–15				135 000–300 000

Platelet adhesiveness according to Hellem s method for whole blood Mean	Frequency distribution			Plasma + ADP		Platelet adhesiveness according to Salzman Frequency distribution		Prothrombin consumption test ( )	Factor VIII ( )
(*)	<20	20–30	>30	0 05 μg/ml	0 10 μg/ml	Low <20	Normal >20		
19	4	5	1	8	21	2	0	20	130
30	1	1	1	16	39	1	0	19	125
24	0	2	0					2	89
27	1	2	1	11	30	1	0	16	100
34	0	0	1					0	
21	3	2	1	13	27	0	2	30	95
13	4	0	0	24	33	1	1	33	70
24	1	4	0	15	30			12	72
34	0	0	1	8	37	0	1		
25	1	2	0	34	51			19	56
26	1	0	2	24	53			22	101
24	1	2	0	19	36			43	69
21	4	6	0	22	46	1	1	26	122
22	1	4	1	20	40	1	0	15	102
25	0	3	1	31	45	0	1	14	71
27	2	2	2	36	66	1	0	27	70
34	0	2	4	36	60	0	1	24	140
35	0	0	1	40		0	1	10	103
34	0	0	1			0	1		
37	0	0	1	23		0	1	18	110
20	5	3	1	34	61	1	1	30	93
35	0	0	1						
20	2	1	2	18	35	0	2	26	54
35	0	0	1			1	0	8	159
41	0	0	1			0	1	16	97
18	4	3	0	10	23	1	0	34	94
24	0	3	0	11	39	0	1	22	
35	0	0	1	27	27	0	1	10	92
19	2	1	0	17	58			33	150
17	2	1	0	13	29			19	56
26	1	2	1			1	0	31	225
23	1	3	0	14	40	1	0	19	83
26	1	1	1	18	35			21	135
45	0	0	1					13	
18	3	1	0	15	39	1	0	43	75
24	0	1	0			0	1	14	
25	0	1	0	15	40	1	0	9	73
0	12	8	7	11	24	1	0	18	80
28	0	1	1	32	53			21	110
29	0	2	0	18	32			9	100
[illegible]6	0	1	0	0	24				
26	0	1	0	24	23	0	1	7	88
17	7	3	0	8	31	2	1	30	84
29	0	1	0	17	21	0	1	9	
37	0	0	1					32	
26	0	1	0	10				9	
24	1	5	2	39	62	3	0	17	118
23	0	1	0			0	1		
[illegible]4	0	1	0	34	41	0	1		
17	3	0	0	19	34	1	0	22	101
18	2	1	0	46	70	1	0	17	115
22	0	1	0					46	69
26	1	5	1	[illegible]9	51	3	2	21	131
35	0	1	2	41	58	1	2	5	147
38	0	0	2	50	73	0	2	21	114
30 S.D. ±4				26 S.D. ±12	48 S.D. ±15			0–30	60–160

supernatant plasma without disturbing the layer of platelets.

Platelet adhesiveness was measured according to a slight modification (7) of Hellem s whole blood method (11) According to this method citrated whole blood is passed through a column of glass beads and the percentage of adherent platelets is calculated Platelet adhesiveness in platelet rich plasma after addition of ADP in various concentrations was determined according to a slight modification (7) of the method of Hellem et al. (12) Platelet adhesiveness was also measured by the original method of Salzman (27) according to which whole blood is allowed to flow through a glass bead filter directly at blood collection

Aggregation of platelets after addition of connective tissue suspension was studied as has earlier been described (8 4) Adhesion of platelets in citrated platelet rich plasma on a glass slide spontaneous aggregation and after recalcification viscous metamorphosis were studied under the phase contrast microscope Aggregation of platelets after addition of various proteolytic enzymes such as thrombin trypsin and papain was studied macroscopically and microscopically as described before (8)

Clot retraction was determined in diluted platelet rich plasma according to a modification of a method used by Voss (8)

The *tourniquet test* was performed by inflating a blood pressure cuff to a pressure intermediate between the systolic and diastolic blood pressure If more than ten blood spots occurred within an area of 20 cm when read after 15 min the test was said to be positive

CASE REPORTS

Family 1

The pedigree is shown in Fig. 1 The results of laboratory studies are summarized in Table I.

K L coord no II 1 female born 1915 Always troubled by menorrhagia necessitating bed rest for the first two days. Four deliveries accompanied by increased bleeding especially after her third parturition. In 1958 several teeth were extracted after which she bled for three weeks and was ultimately hospitalized Her haemoglobin had then fallen to 9 g per 100 ml. She bruised readily she often has gingival bleeding and has prolonged

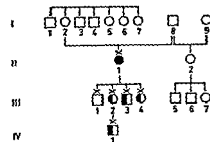


Fig 1 Family 1

Symbols used in the pedigrees ☐ male ○ female ■ ● affected patients ◧ ◐ individuals suspected of being affected × above sex symbol indicates that the individual has been investigated ◇ descendants of minor interest

bleeding after even small cuts Owing to her severe menstruations she was roentgen-castrated She has only received two blood transfusions

Laboratory studies The patient has been followed up since 1963 The Ivy bleeding time almost always exceeded 30 min. Platelet adhesiveness determined by Hellem s whole blood method his plasma ADP method and Salzman s method was decreased Coagulation factors including factor VIII were always normal The prothrombin consumption test and clot retraction were also normal On direct microscopical examination the platelets appeared normal At examination under the phase contrast microscope adhesion and aggregation of her platelets in platelet rich citrated plasma were abnormally weak. Aggregation occurred after addition of ADP or connective tissue suspension. Viscous metamorphosis was normal

Therapeutical trials Administration of 600 ml stored plasma 900 ml fresh blood 1400 ml platelet rich plasma containing 450 10^9 platelets, or one dose of fraction I-0 had no effect on the bleeding time or platelet adhesiveness Prednisone administered in a dose of 5 mg three times a day for three weeks produced no effect either Epsilon amino-caproic acid in a dose of 6 g four times a day decreased the amount of blood lost at menstruation.

G L coord no III 2 female born 1944 Ready bruisability Occasional nose bleeding Prolonged bleeding after cuts. Copious menstrual flow

Laboratory studies Ivy bleeding time prolonged 15-20 min Platelet adhesiveness was normal or decreased according to Hellem s whole blood method and decreased according to Salzman s method.

G L coord no III 3 male born 1946 Prolonged bleeding after cuts Occasional nose bleeding

Laboratory studies The patient was investigated twice On the first occasion he had a prolonged Ivy bleeding time of 21 min on the second of only 12 min. Platelet adhesiveness by Hellem s whole blood method bordered on the lower normal limit

E L coord no III 4 female born 1955 Ready bruisability and occasional nose bleeding and gingival bleeding. Prolonged bleeding after cuts Bled for more than 4 hours after extraction of a deciduous tooth. Ear bled for several days during otitis

Laboratory studies Bleeding time according to Ivy varied between 10 and 26 min. The platelet adhesiveness also varied widely

J A coord. no IV 1 male born 1965 Large haematoma after triple vaccination Ivy bleeding time 13 min.

B L coord. no III 1 male born 1943 No bleeding symptoms. Normal laboratory findings

Comments

The proband K L coord no II 1 had a haemorrhagic diathesis with menorrhagia and bleeding after tooth extractions The Ivy bleeding time was prolonged but there were no signs of von Willebrand s disease Platelet adhesiveness was

decreased Three of her four children and one grandchild had a mild bleeding tendency and a variable but mostly prolonged Ivy bleeding time as well as a variable but mostly decreased platelet adhesiveness—they probably had the same disease but in an even milder form owing to a weaker expressivity

Family 2 (Pedigree in Fig 2)

L. O coord. no III 5 male born 1957 Often had nose bleeding Bruised readily Prolonged bleeding after extraction of deciduous teeth necessitated surgical intervention on the following day Occasional rectal bleeding Tonsillectomy followed by profuse bleeding with fall of haemoglobin from 13 g per 100 ml to 9 g per 100 ml.

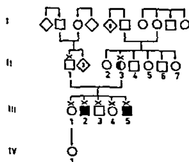
Laboratory studies Ivy bleeding time prolonged and often exceeded 30 min. Platelet adhesiveness according to Hellem s whole blood method and plasma ADP method decreased Adhesiveness examined twice by Salzman s method was normal. Prothrombin consumption test normal or slightly abnormal Aggregation with ADP or connective tissue suspension was normal Spontaneous aggregation occurred. Normal clot retraction and viscous metamorphosis Normal coagulation factors including factor VIII No abnormal fibrinolysis

I O coord. no III 2 male born 1946 Brother of L O Seldom bruises. Prolonged bleeding after cuts Bled for three days after extraction of deciduous teeth.

Labo atory stud es Prolonged Ivy bleeding time mostly exceeding 30 min Platelet adhesiveness according to Hellem s whole blood method was decreased on all four occasions. Platelet adhesiveness estimated by Hellem s plasma ADP method and Salzman s method was also weak. No other platelet abnormality demonstrable Normal factor VIII as well as other coagulation factors

I O coord no II 3 female born 19 2. No bleeding symptoms

Laboratory stud es Ivy bleeding time prolonged (17–2. min) Platelet adhesiveness, as determined by Hellem s whole blood and plasma ADP methods was slightly decreased



F g Family ..

I O coord no III 1 female born 1944 Sister of L. O Ready bruisability and profuse menstrual bleeding

Laboratory studies Normal Ivy bleeding time Platelet adhesiveness according to Hellem s whole blood method weak to normal Factor VIII was 56

A B O coord no III 4 female born 1949 Sister of L O Ready bruisability and profuse menstrual bleeding Massive bleeding after pontaneous abortion

Laboratory studies Ivy bleeding time and platelet adhesiveness variable often normal

B O coord no III 3 male born 1958 Gingival bleeding only symptom

Labo atory studies Ivy bleeding time 10–17 min. Platelet adhesiveness according to Hellem s whole blood method bordered on the lower limit of the normal range

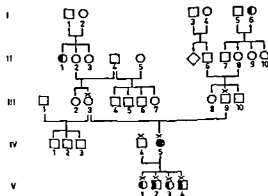
Findings in the father of L. O were normal

Comments

The proband L O coord no III 5 had a haemorrhagic diathesis with prolonged bleeding time and decreased platelet adhesiveness One of his four siblings had the same syndrome while the others were probably healthy Their father was healthy while their mother had a prolonged Ivy bleeding time and slightly decreased platelet adhesiveness but no bleeding symptoms

Family 3 (Pedigree in Fig 3)

H M coord no IV 5 female born 1933 At times ready bruisability and nose bleeding Copious menstrual bleeding necessitating bed rest the first few days. Neither appendectomy in 1947 nor extraction of teeth in 1949 was complicated by undue bleeding Operated upon in 1958 for uterine prolapse and then bled profusely One of three spontaneous abortions was complicated by severe bleeding. Has had four children without undue loss of blood Her menstrual bleedings have responded well to



F g 3 Family 3

supernatant plasma without disturbing the layer of platelets

Platelet adhesiveness was measured according to a slight modification (7) of Hellem s whole blood method (11) According to this method citrated whole blood is passed through a column of glass beads and the percentage of adherent platelets is calculated Platelet adhesiveness in platelet rich plasma after addition of ADP in various concentrations was determined according to a slight modification (7) of the method of Hellem et al (12) Platelet adhesiveness was also measured by the original method of Salzman (27) according to which whole blood is allowed to flow through a glass bead filter directly at blood collection

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Laboratory studies The patient has been followed up since 1963 The Ivy bleeding time almost always exceeded 30 min Platelet adhesiveness determined by Hellem s whole blood method his plasma ADP method and Salzman s method was decreased Coagulation factors including factor VIII were always normal The prothrombin consumption test and clot retraction were also normal On direct microscopical examination the platelets appeared normal At examination under the phase contrast microscope adhesion and aggregation of her platelets in platelet rich citrated plasma were abnormally weak. Aggregation occurred after addition of ADP or connec tive tissue suspension *Viscous metamorphosis was normal.*

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Laboratory studies Ivy bleeding time prolonged 15- 0 min Platelet adhesiveness was normal or decreased ac cording to Hellem s whole blood method and decreased according to Salzman s method.

G L coord no III 3 male born 1946 Prolonged bleeding after cuts Occasional nose bleeding

Laboratory studies The patient was investigated twice On the first occasion he had a prolonged Ivy bleeding time of 21 min on the second of only 12 min Platelet adhesiveness by Hellem s whole blood method bordered on the lower normal limit

E L coord no III 4 female born 1955 Ready bruisability and occasional nose bleeding and gingival bleeding Prolonged bleeding after cuts Bled for more than 24 hours after extraction of a deciduous tooth Ear bled for several days during otitis

Laboratory studies Bleeding time according to Ivy varied between 10 and 26 min. The platelet adhesiveness also varied widely

J A coord no IV 1 male born 1965 Large haematoma after triple vaccination Ivy bleeding time 13 min.

B I coord no III 1 male born 1943 No bleeding symptoms Normal laboratory findings

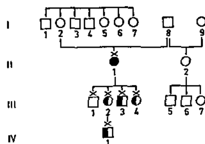


Fig 1 Family 1

Symbols used in the pedigrees ☐ male ○ female ■ ● affected patients ◧ ◐ individuals suspected of being affected × above sex symbol indicates that the individual has been investigated ◇ descendants of minor interest

Comments

The proband K L coord no II 1 had a haemorrhagic diathesis with menorrhagia and bleeding after tooth extractions The Ivy bleeding time was prolonged but there were no signs of von Willebrand s disease Platelet adhesiveness was

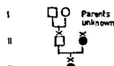


Fig 5 Family 5

Comments

The boy J W coord no III 2 had haemorrhagic diathesis with a constantly prolonged Ivy bleeding time and decreased platelet adhesiveness His sister has possibly a similar haemorrhagic diathesis and his mother had the same though less pronounced abnormalities

Family 5 (Pedigree in Fig 5)

E N coord no III 1 female born 1954 Bruises easily Frequent nose bleeding Extractions of deciduous teeth uncomplicated Profuse menstrual bleeding Occasional rectal bleeding

Laboratory studies Prolonged Ivy bleeding time exceeding 30 min. Decreased adhesiveness as tested by Hellem s whole blood and plasma ADP methods and by Salzman s method Adhesion to glass and spontaneous aggregation occurred Platelets aggregated after addition of ADP or connective tissue suspension Normal viscous metamorphosis and clot retraction Normal prothrombin consumption and normal coagulation factors including factor VIII

B N coord no II 2 female born 1927 Mother of E. N Bruises easily Profuse menstrual bleeding. Otherwise no bleeding symptoms

Laboratory studies Ivy bleeding time often prolonged 18-20 min Platelet adhesiveness borders on lower limit of normal range as tested by Hellem s whole blood method Hellem s plasma ADP method and Salzman s method

The father of E N was healthy B N was an orphan and did not know anything about her biological parents.

Comments

The patient E N coord no III 1 had marked bleeding symptoms prolonged Ivy bleeding time and decreased platelet adhesiveness In her mother the bleeding time was only moderately prolonged and platelet adhesiveness bordered on the lower limit of the normal range

Family 6 (Pedigree in Fig 6)

M J coord no III 2, male born 1959 Occasional microscopic haematuria Bruises easily Frequent nose bleeding.

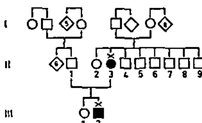


Fig 6 Family 6

Laboratory studies Ivy bleeding time 12-20 min Platelet adhesiveness as determined by Hellem s whole blood method decreased Normal coagulation factors including factor VIII

S J coord no II 3 female born 1931 Mother of M J Bruises easily Nose bleeding common in childhood Often had gingival bleeding Bled much at parturitions but did not require blood transfusions No undue bleeding at operation for varicose veins or extraction of teeth

Laboratory studies Ivy bleeding time 15-25 min Decreased platelet adhesiveness as measured by Hellem s whole blood method

Comments

The patient M J had mild bleeding symptoms moderately prolonged Ivy bleeding time and low platelet adhesiveness His mother had less pronounced symptoms but similar laboratory findings

Family 7 (Pedigree in Fig 7)

O S coord no II 2 male born 1962 Abrasion of the pharyngeal tonsil without immediate bleeding but one week later massive haemorrhage occurred and he was readmitted in a state of shock and was treated with dextran and blood transfusions. Was sent home a few days later but after a week bleeding recurred and he again fell into a state of shock.

Laboratory studies Moderately prolonged Ivy bleeding time 15-30 min Platelet adhesiveness as measured with Hellem s whole blood method variable but often decreased Salzman s test decreased Spontaneous aggregation occurred Aggregation after addition of ADP and connective tissue suspension was normal Normal viscous metamorphosis and clot retraction Normal coagulation factors including factor VIII Prothrombin consumption test normal

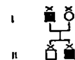


Fig 7 Family 7

S S coord. no I 1 born 1936 Father of O S Nose bleeding in childhood but otherwise no bleeding symptoms

Laboratory studies Prolonged Ivy bleeding time once exceeding 30 min Moderately low adhesiveness as measured by Hellem s whole blood and plasma ADP methods and Salzman s method Prothrombin consumption test and factor VIII normal Platelets aggregated with ADP and connective tissue suspension

The mother and one brother of O S were also studied They had no bleeding symptoms and the laboratory findings were normal

Comments

The patient O S coord no II 2 had severe bleeding episodes following surgery Ivy bleeding time was prolonged and the platelet adhesiveness was often decreased His father had hardly any bleeding symptoms but prolonged bleeding time and decreased platelet adhesiveness Other relatives healthy

Family 8 (Pedigree in Fig 8)

I B coord no II 3 female born 1912 Bruises easily Occasional nose bleeding Haematuria on one occasion Prolonged bleeding once massive after tooth extractions Profuse menstrual bleeding Normal deliveries Bled heavily after a spontaneous abortion. In 1932 and 1944 operated upon for goitre without undue bleeding but profuse bleeding after similar operation in 1952 In 1965 a large haematoma developed in her thigh and later bleeding in her left knee joint

Laboratory studies Prolonged Ivy bleeding time 18–25 min Platelet adhesiveness as measured by Hellem s whole blood method and Salzman s method was decreased Variable adhesiveness by Hellem s plasma ADP method Spontaneous aggregation occurred. Normal aggregation with ADP and connective tissue suspension. Normal clot retraction and viscous metamorphosis Normal coagulation factors Prothrombin consumption test often abnormal

O B coord no III 2 male born 1944 Son of I B Bled for a week after tonsillectomy and had to be re operated upon 4 times

Laboratory studies Ivy bleeding time 15 min. Platelet adhesiveness 24% as measured by Hellem s whole blood method which is in the lower limit of the normal range Adhesiveness as measured by Salzman s method normal Normal prothrombin consumption test and factor VIII

B B coord no III 1 male born 1937 No bleeding symptoms

Laboratory studies Normal Ivy bleeding time but platelet adhesiveness bordered on lower normal limit.

Comments

The patient I B had mild bleeding symptoms prolonged bleeding time and decreased platelet adhesiveness She had two sons one of whom had bled after tonsillectomy Bleeding time and platelet adhesiveness were not abnormal with certainty

Family 9 (Pedigree in Fig 9)

A A coord no II 1 female born 1931 Always marked tendency to bruise Prolonged bleeding after cuts An intramuscular injection once produced a large haematoma Prolonged bleeding after tooth extractions Frequent nose bleeding In 1958 hysterectomy because of severe menorrhagia without undue bleeding Three uncomplicated deliveries Under cover of EACA the patient was operated upon in 1966 with resection of the colon because of a polyp Her bleeding symptoms were largely periodical at times she had no trouble but at other times she bruised readily and often suffered from nose bleeding

Laboratory studies From 1953 the patient was followed closely and has been investigated completely more than 100 times Her Ivy bleeding time was prolonged often exceeding 30 min The Duke bleeding time was normal or slightly prolonged Platelet adhesiveness as measured by Hellem s whole blood and plasma ADP methods and by Salzmon s method was usually decreased Factor VIII as well as other coagulation factors were normal Spontaneous adhesion to glass and aggregation in platelet rich citrated plasma occurred The platelets aggregated after addition of ADP or connective tissue suspension Viscous metamorphosis and clot retraction were normal Normal prothrombin consumption

Therapeutical trials On five occasions the patient received 400 to 800 ml of fresh plasma without normaliza

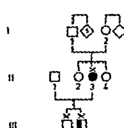


Fig 8 Family 8

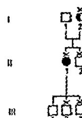


Fig 9 Family 9

tion of the bleeding time or platelet adhesiveness She also received 500 ml of 20 fat emulsion Intralipid® which had been found to increase the platelet adhesiveness in normals and was therefore tried in this patient A rise in the adhesiveness occurred also in this patient but the bleeding time was unchanged (5) Epsilon amino-caproic acid in a dose of 6 g three times a day or AMCA, amino-methyl cyclohexane carboxylic acid 2.5 g four times a day decreased the severity of her bleeding symptoms but had no effect on the bleeding time

G L coord. no I 2 female born 1906 Mother of A A No bleeding symptoms

Laboratory studies Ivy bleeding time slightly prolonged 18 min Variable platelet adhesiveness as measured by Hellem s methods Normal factor VIII

One brother and one son of the proband A A had bled profusely after tooth extraction but bleeding time and platelet adhesiveness as measured by the methods used were normal Both were therefore regarded as healthy

Comments

A patient with pronounced bleeding tendency prolonged bleeding time and mostly low platelet adhesiveness The severity of the condition varied periodically as did the laboratory findings The corresponding laboratory values for the mother bordered on the lower limit of the normal range

Family 10 (Pedigree in Fig 10)

E B coord no III 2 female born 19?3 Ready bruisability was a major complaint. Profuse menstrual bleedings Large haematomas developed after intramuscular injections. Copious bleeding after tooth extractions

Laboratory studies Prolonged Ivy bleeding time often exceeding 30 min. Decreased platelet adhesiveness as measured by Hellem s methods for whole blood and plasma ADP as well as by Salzman s method Normal prothrombin consumption Adhesion to glass and spontaneous aggregation occurred in platelet rich citrated plasma. The platelets aggregated after addition of ADP or connective tissue suspension Normal clot retraction and viscous metamorphosis Factor VIII as well as other coagulation factors were normal

E H coord no II 2 female born 1891 Mother of E B No bleeding symptoms but operated upon twice in 1967 because of hip fracture and then received two pints of blood

Laboratory studies Prolonged bleeding time Platelet adhesiveness as measured by Hellem s whole blood method bordered on the lower limit of the normal range

E B had one daughter and one granddaughter Neither had bleeding symptoms and the laboratory findings were normal in both

Comments

The patient E B coord no III 2 had pronounced bleeding symptoms prolonged bleeding time and decreased platelet adhesiveness She had been referred to us once several years previously but her prolonged bleeding time on that occasion was ignored and she was considered healthy Later her bleeding symptoms prompted reinvestigation and her condition was then diagnosed as mild thrombasthenia She had no siblings but one child who was healthy Her mother has a moderately prolonged Ivy bleeding time and may have the same disorder but milder

Family 11 (Pedigree in Fig 11)

D H coord no II 1 born 1913 Bruises easily No tendency to nose bleeding but often has gingival bleeding. Prolonged bleeding after cuts Hospitalized twice because of severe menorrhagia and was then admitted in a state of shock and given blood transfusions Hysterectomy was therefore performed without undue bleeding Referred to our laboratory for investigation because of prolonged bleeding after tooth extractions

Laboratory studies The Ivy bleeding time was constantly found to be prolonged Platelet adhesiveness as measured by Hellem s whole blood method was variable but often decreased and repeated examination by Salzman s method showed no adhesiveness at all. But it was normal according to Hellem s plasma ADP method. Spontaneous aggregation occurred and her platelets ag

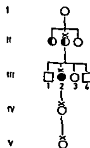


Fig 10 Family 10

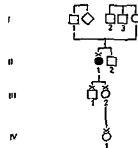


Fig 11 Family 11

gregated also after addition of ADP or connective tissue suspension Viscous metamorphosis and clot retraction were normal Factor VIII and coagulation factors were normal

Her parents were dead her two children had no bleeding symptoms and the laboratory findings were not with certainty abnormal

Comments

Patient with menorrhagia and prolonged bleeding after tooth extractions Constantly prolonged bleeding time and decreased platelet adhesiveness

Family 12

A O male born 1938 Often bruises and occasionally has large haematomas Nose bleeding common in childhood Frequent gingival bleeding Prolonged bleeding after cuts In 1958 a large haematoma developed after an operation of the hip In 1960 severe bleeding requiring blood transfusions after tonsillectomy

Laboratory studies Ivy bleeding time slightly prolonged 13–22 min with a mean of 18 min Always markedly decreased platelet adhesiveness as measured by Hellem s whole blood method and Salzman s method Factor VIII and other coagulation factors were normal The results of the prothrombin consumption varied Platelet factor 1 was low despite normal level of factor V in plasma Platelet factors 3 and 4 were normal Spontaneous aggregation of platelets in citrated plasma on a glass slide occurred Normal aggregation with ADP or connective tissue suspension Normal viscous metamorphosis and clot retraction

The patient knows of no haemorrhagic diathesis in his family and no other member was available for testing

Comments

Patient with bleeding tendency moderately prolonged bleeding time but markedly decreased platelet adhesiveness

Family 13 (Pedigree in Fig 12)

I F coord no II 3 female born 1928 Always bruised easily In 1951 operated on for hernia without undue bleeding In 1958 parturition without bleeding In 1963 Caesarean section because of placenta praevia. Massive bleeding during operation and large wound haematomas afterwards

Laboratory studies Prolonged Ivy bleeding time Decreased platelet adhesiveness as determined by Hellem s whole blood method and Salzman s method but not by Hellem s plasma ADP method Normal prothrombin consumption and coagulation factors Platelets in platelet rich citrated plasma adhe ed on a glass slide and aggregated Aggregation by ADP or connective tissue suspension was normal

Comments

A patient with a bleeding tendency especially after surgery with prolonged Ivy bleeding time and decreased platelet adhesiveness

Family 14

E S female born 1958 Often bruises On three occasions severe nose bleeding Once profuse bleeding after extraction of deciduous teeth

Laboratory studies Ivy bleeding time exceeding 30 min Decreased platelet adhesiveness by Hellem s whole blood method No known haemorrhagic diathesis in her family which was not investigated

Comments

Patient with mild bleeding symptoms prolonged bleeding time and decreased platelet adhesiveness

Family 15 (Pedigree in Fig 13)

C H coord no III 2 female born 1949 Often profuse menstrual bleeding Once fell into shock because of severe menorrhagia and was given several pints of blood Bleeding two weeks later lasted for three weeks Otherwise no bleeding symptoms

Laboratory studies Prolonged Ivy bleeding time exceeding 30 min Often decreased platelet adhesiveness as tested by Hellem s whole blood method and Salzman s method but normal by Hellem s plasma ADP method Spontaneous platelet aggregation occurred ADP or connective tissue suspension aggregated platelets in platelet rich citrated plasma Normal viscous metamorphosis and

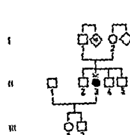


Fig 12 Family 13

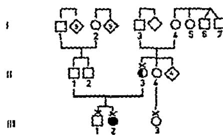


Fig 13 Family 15

clot retraction. Normal prothrombin consumption Normal factor VIII and other coagulation factors

M H., coord no II 3 female born 1918 Mother of C H Easily bruises and frequent nose bleedings in childhood. Prolonged bleeding after tooth extraction when 19 years old Profuse bleeding after tonsillectomy in 1936 Curettage in 1946 and 1949 because of profuse menstrual bleeding

Laboratory stud es Ivy bleeding time was slightly prolonged. Platelet adhesiveness as tested by Hellem s whole blood and plasma ADP methods normal and variable by Salzman s method

The b other of C H had no bleeding symptoms and was found to be healthy

Comments

The patient C H had menorrhagia prolonged bleeding time and decreased platelet adhesiveness Her mother had bleeding symptoms but less marked laboratory abnormalities

DISCUSSION

The investigation revealed a number of patients with tendency to ready bruisability nose bleeding prolonged bleeding after tooth extractions and surgery and in the females menorrhagia In most patients the symptoms fluctuated periodically in severity The Duke bleeding time was mostly normal The Ivy bleeding time was prolonged sometimes only to 15–20 min but most often it exceeded 30 min The platelet count was normal Platelet adhesiveness as tested by Hellem s or Salzman s methods was mostly decreased Prothrombin consumption was normal in most patients When a drop of platelet rich citrated plasma was placed on a glass slide under a cover glass slight movements of the plasma invariably caused the platelets to adhere and to spread on the glass and also to aggregate Aggregation was nevertheless generally weak and retarded The platelets r gularly aggregated after addition of ADP or connective tissue suspension Clot retraction was normal. The coagulation factors were normal and factor VIII was not decreased No pathologic fibrinolysis was demonstrated

Two patients were treated with fresh plasma and one with fraction I–0 without normalization of the bleeding time

Of the patients referred to us five were males and ten females Of typical cases detected at familial investigation two were males and five females A further five males and six females were probably affected The increased incidence of females can be largely explained by the fact that menorrhagia was a common complaint necessitating investigation

Family investigation often revealed that other relatives such as siblings one of the parents or children had a similar but usually milder form of the syndrome The results were not so clearcut as in a family described previously with a similar but different disease (6) In family 4 the patient and his mother had an Ivy bleeding time of usually more than 30 min In family 2 two brothers and their mother were affected In families 1 and 3 several children had a milder bleeding tendency than their mothers and frequently but not constantly a prolonged Ivy bleeding time and decreased platelet adhesiveness No abnormality was found in the elderly parents of patient H M in family 3 Also in other families a dominant mode of inheritance was likely or possible but the penetrance was weak. At least periodically in many probable carriers of the gene the bleeding time and platelet adhesiveness were within normal limits

The haemorrhagic diathesis of the affected patients was probably caused by a primary platelet defect responsible for the decreased adhesiveness and prolonged bleeding time The long history of recurrent bleeding episodes in many patients the family history and the absence of systemic disease or drug intake argue for a congenital disorder and against an acquired condition Before deciding on the diagnosis other bleeding disorders must be excluded

The most important differential diagnosis was von Willebrand's disease This condition has similar bleeding symptoms with a prolonged bleeding time and mostly decreased platelet adhesiveness as measured by Salzman s method (4 27) Deficiency of factor VIII is a specific and obligatory sign of von Willebrand s disease (17) but was normal in these patients In von Willebrand s disease the adhesiveness was found to be normal when measured by Hellem s whole blood method (7) Three patients in our study received infusions of fresh plasma or fraction I–0 without any effect on the bleeding time which argues against von Willebrand s disease The differential diagnosis was important since bleeding in patients with von Willebrand s disease should be treated with fresh plasma or plasma derivatives (17) which is useless

in patients with this disease A low platelet adhesiveness as estimated by Salzman s method could however not differentiate between the two conditions

The disease was not identical with Glanzmann s severe thrombasthenia which according to the modern concept is characterized by complete absence of aggregation with ADP (8 9)

The patients in this study resembled the patients in another family with 16 affected members whose disease was called moderately severe thrombasthenia (6) In that family the heredity was typically dominant and the findings more constant Besides prolongation of the bleeding time and decreased adhesiveness the platelets in that family did not aggregate spontaneously and they swelled in a peculiar way when suspended in citrate

None of the patients showed the giant platelets present in the disease first described by Bernard and Soulier (1) as dystrophie thrombocytaire hemorragipare congenitale

Patients with mild bleeding symptoms and often other weak abnormalities of various types have been described by other investigators and in these patients the disease may be similar to or identical with that in our patients Thus patients with prolonged bleeding time as single symptom have been described by Jacobson (15) In a survey of French patients with bleeding disorders Larrieux (16) reported 19 patients with a prolonged bleeding time the only abnormality found and these patients may belong to this group Hirsh et al (13) described a patient who bruised readily and in whom investigation showed a prolonged bleeding time and complete absence of aggregation of the platelets after addition of connective tissue suspension This patient evidently differed from our patients who did not show any qualitative abnormality by this test Under the name of Portsmouth syndrome O Brien (26) described ten patients with mild bleeding symptoms a prolonged bleeding time decreased platelet adhesiveness and an abnormal reaction with connective tissue suspension Hirsh et al (14) found it difficult to determine the normal range of the connective tissue platelet reaction because the activity of the connective tissue extract varied from batch to batch and decreased on storage Consequently they considered the reaction to be abnormal only when aggregation could not be demonstrated at all with a very active connective tissue extract and this is the view we have adopted Hardisty and Hutton (10) described 13 patients with mild bleeding tendencies in whom a normally rapid platelet aggregation on addition of ADP was followed by unusually rapid disaggregation Platelet aggregation in response to addition of collagen suspensions in vitro was impaired and platelet adhesiveness to glass was mostly decreased Weiss (28) described a bleeding disorder in six women characterized by a defective availability of platelet factor 3 and defective release of aggregating activity from the platelets on incubation with kaolin The patients of Hardisty and Hutton and of Weiss were regarded as belonging to the group of thrombopathy As the prothrombin consumption test was normal in most of our patients and when tested platelet factor 3 was also normal we hesitated to call the condition thrombopathy

An interesting observation made in a previous investigation (8) was that many of the close relatives of three patients with severe thrombasthenia of Glanzmann s type had similar mild bleeding symptoms a prolonged Ivy bleeding time and decreased platelet adhesiveness just as the patients in this study These relatives were probably carriers of the gene for severe thrombasthenia in a single dose It is therefore possible that the haemorrhagic diathesis as well in this study was due to a platelet defect and that the patients are carriers of the gene for severe thrombasthenia of Glanzmann s type but in a single dose This would lend further support to the diagnosis of mild thrombasthenia

There is no specific treatment for the condition As local fibrinolysis is physiologically present treatment with epsilon amino-caproic acid often decreases the bleeding in association with surgery or gynaecological disorders Menorrhagia also responded favourably to treatment with gestagens Thorough haemostasis is necessary during operations Neither plasma nor plasma derivatives have proved to be of any use When blood is used it should preferably be fresh

ACKNOWLEDGEMENTS

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Universite de Paris Faculte de Medecine Chaire de Clinique Nephrologique Hopital Necker, Professeur J Hamburger, 149 rue de S vres Paris 15 *Cours de Perfectionnement sur la Nephrologie* les lundi 6 mardi 7 et mercredi 8 mai 1968

Il est recommande de s inscrire assez a l avance le nombre des participants etant limite Pour tous renseignements s adresser au secretariat du Professeur Agrege J Crosnier Hopital Necker 149 rue de S vres Paris 15ᵉ

The Third International Meeting of Endocrinology will be held in Marseilles May 9 to 12 1968

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There will be sessions on three topics (metabolism of red cells membrane permeability of red cells and platelets metabolism of thrombocytes) each of which shall be opened by main lectures and special reviews The remaining time will serve for presentation of short papers and an extensive discussion

Enquiries are invited and should be directed to the Secretariat of the First International Symposium on metabolism and membrane permeability of erythrocytes and thrombocytes Wiener Medizinische Akademie Stadiongasse 6–8 1010 Vienna Austria

The Fifth European Congress of Cardiology will be held in Athens September 8 to 14 1968

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Secretariat 24 Ravine St Athens 140 Greece The Congress Secretariat will be located at the Zappeion Hall during the Congress

The Third International Symposium on Drugs Affecting Lipid Metabolism will be held in Milan Italy September 9–11 1968

For information and forms contact Miss Has l J Prain Secretary to the Organizing Committee Institute of Pharmacology Via A del Sarto 21 20129 Milan Italy

The Tenth International Congress on Diseases of the Chest will be held in the Washington Hilton Hotel Washington D C USA October 4 to 8 1968

For information and registration forms write the Am rican College of Chest Physicians 112 East Chestnut Street Chicago Ill 60611 USA

The Third International Conference on Congenital Malformations will be held in the Netherlands Congress Centre in The Hague S ptember 8 to 12 1969

For information write the local secretariat c/o Holland Organizing Centre 16 Lange Voorhout The Hague The Netherlands

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Acta med scand Vol 183 pp 177–182 1968

A RETROSPECTIVE STUDY OF COMPLICATIONS FOLLOWING NEPHROANGIOGRAPHY CARDIOANGIOGRAPHY AND CORONARY ANGIOGRAPHY

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Fron the Department of Med cıne Karol nska Instıtutet at Serafıme lasarettet Stockholm Sweden

Abst act A study ıs presented of 3 9 records of patıents on whom an ography had been performed

Fifteen complıcatıons were found (4 6) These are listed ın Table IV

The five complıcatıons occurrıng ın connectıon wıth nephroangıographies performed ın the ınvestıgatıon of hypertensıon are assessed against the benefit obtaıned Only three patients out of 101 ınvest gated have deri ed any benefit from the resultıng operatıon

Some of the lıterature ıs revıewed and the authors conclude that the ındıcatıons for nephroangıography ın the ınvestıgatıon of hypertensıon mıght be modified by the ınclusıon of other selectıve ınvestıgatıons

Complıcatıons following arterıal angıographıes are to some extent unavoıdable An apparent ın crease of complıcatıons ın connectıon with dıag nostıc nephroangıograms coronary angıograms and cardıoangıographıes was observed ın the Medıcal Department of the Seraphımer Hospıtal durıng 1965 Thıs observatıon lead to the present retrospectıve study the aım of whıch was to make a clınıcal evaluatıon of these radıologıcal procedures The rısk for the nephroangıograms was further compared with theır benefit as expressed by the ınstances ın whıch the findıngs ınıtıated surgıcal therapy

MATERIAL

The records of 3 9 pat ents who had been ın est ated with arterıal angıographıes on account of hypertens e renal or card ac d sease have been studıed Age and sex d strıbut on are shown ı Table I

1 Nephroangıographıes

At the request of the med cal department 5 nephroangıographıes were performed by the X ray department durıng the 8 year perıod 1958–1965 The case records of 212 were traced and re iewed One hundred and one of these ınvestıgatıons were for hypertensıon and 53 were carrıed out on cases of suspected renal tumour The re maınıng 58 were made ın the ın estıgatıon of other nephropathıes Accordıng to the records sıx of the 12 examınatıons were followed by complıcatıons and five of these were carrıed out as part of an ınvestıgatıon for hypertensıon The Seldınger technıque (2_) was used ın all cases except two ın whıch a translumbar puncture was chosen No complıcatıons occurred ın these

Case repo ts of complıcatıons following neph oangıog aphy

1 Woman aged 73 ınvestıgated for malıgnant medıastınal tumour Followıng the examınatıon she developed a large haematoma ın the punctured gro n whıch was absorbed ın a couple of weeks

2 Man aged 50 ınvestıgated for hypertensıon Thıs patıent had shown sıgns of cırculatory shock followıng an ıntravenous pyelography sıx days before the nephroangıogram whıch also was followed by cırculatory shock and precordıal paın He quıckly recovered with approprıate treatment and no dısabılity followed ECG tracıngs and SGOT and SGPT levels showed no evıdence of myocard al dama e

3 Man aged 53 ınvestıgated for hypertensıon Followıng nephroangıography the patıent de eloped a haematoma about one ınch ın dıameter ın the punctured groın accompan ed by a rısın t mperature He rapıdly ımproved wıth penıcıllın and experıenced no dısab lıty thereafter

4 Woman aged 52, ınvestıgated for hypertensıon Followıng the nephroang ography an arterıal aneurysm developed ın her left gro n whıch was operated on sıx months later ın two separate sessıons Afterwards the patıent complaıned of a cold feelıng n her left foot She had no ıntermıttent claud catıon but the arterıal pulsatıons ın her left foot were weaker than on the rıght Thıs patıent also had chronıc pyelonephrıtıs was an alcohol and dru add ct and suffered from Adams-Stokes attacks

5 Woman aged 35 nvestıgated for hypertensıon In 1959 she had prolonged bleedıng from the s te of punc

Table I *Distribution of age and sex at angiographies*

	10–19	20–29	30–39	40–49	50–59	60–69	70–79	Σ	♂	♀
All angiographies	9	23	38	93	101	49	16	329	203	1 6
Nephroangiography (total)	2	13	26	52	61	42	16	212	122	90
Nephroangiography in the investigation of hypertension	1	8	13	32	29	12	2	101	52	49
Cardioangiography	7	9	7	17	6	—	—	46	26	0
Coronary angiography	—	1	5	24	34	7	—	71	55	16
Complications	—	1	3	5	5	—	1	15	6	9

ture following a carotid angiogram Following the nephroangiography she developed an aneurysm in her groin It was operated on three days later and a still patent arterial puncture wound was found A large haematoma was evacuated requiring an incision of 20 cm after which complete recovery followed

6 Woman aged 25 investigated for hypertension Three hours following the nephroangiography the patient was found to be pulseless in her punctured leg An angio graphy confirmed an occlusion of the popliteal artery The patient was treated with streptokinase for three days during which she developed a temperature and moderate haematomas formed in the groins She quickly recovered and was discharged one week later with no remaining symptoms

In the group of 101 patients investigated for hyperten sion we also studied the extent to which the investiga tion lead to an operation which was of benefit to the patient In 25 cases abnormal findings of the renal ar terial structure had been found (Table II) In 18 of these no operation was performed either because of complicating disease such as general atherosclerosis or bilateral renal disease or as the findings were not con sidered to be the cause of the hypertension. In the remaining seven cases either a reconstructive operation on the renal artery or a nephrectomy was performed

Table II *Pathological findings at 101 nephroangio graphies performed in investigation of hypertension*

	No
Unilateral stenosis	7
Minimal unilateral stenosis	4
Unilateral stenosis + hypoplasia	2
Unilateral stenosis + contracted kidney	1
Total unilateral occlusion	2
Bilateral stenosis	2
Minimal bilateral stenosis	1
Aplasia	1
Contracted kidney	1
Small anomalies of the renal artery	2
Aneurysm of the renal artery	2
Total	25

Case reports on patients operated on for renal artery stenosis

1 Man aged 21 with marked hypertension (?0/110) and a stenotic lesion at the origin of the left renal artery A reconstructive graft operation was performed and the patient has been normotensive (1?5/80) during the following five years without further treatment An angiography was repeated six months after the operation and revealed a minimal stenosis at the same site

2 Woman aged 5? with a past history of preeclampsia in 1947 followed by persistent hypertension Nephro angiography in 196_ showed total occlusion of the right renal artery A right sided nephrectomy was performed The patient s blood pressure while on treatment before the operation had been 210/110 Following operation but with the same treatment the blood pressure fell to 160/90 associated with marked subjective improveme A follow up examination five years later again showed a blood pre sure of 160/90

3 Man aged 61 hypertensive since 1944 and with a myocardial infarction in 1956 In 196? n phroangio graphy revealed marked steno is with a poststenotic dila tation of the left renal artery A reconstructive graft operation was carried out the following year Angio graphy after the operation showed marked widening of the left renal artery with no stenosis The operation had no effect on the blood pressure which remained un changed at 210/110 with unaltered therapy

4 Woman aged 60 hypertensive and with a history of pyelonephritis intermittent claudication diabetes and myocardial infarction Nephroangiography in 1960 re vealed marked stenosis of the right renal artery with a contracted kidney In 1962 a right sided nephrectomy was performed without any effect on the blood pressure The stenosis was shown to be due to a perinephritic in flammation

5 Woman aged 53 with repeated urinary infections and hypertension for at least three years Nephroangio graphy in 196 showed an occluded left renal artery A left sided nephrectomy was performed The patient s pre operative blood pressure was ?40/135 in spite of treat ment with hydralazine and diuretics During follow up for three years the blood pressure has been 150/100 with diuretics only There was also considerable subjective impro ement

6 Man aged 45 previously in good health Hyperten sion was accidently discovered in 196? and a nephro angiography revealed moderate stenosis and a poststenotic

dilatation of the right renal artery. In 1963 an aortorenal bypass was performed. An angiography after the operation revealed a normal vascular pattern to both kidneys. There was, however, no effect on the blood pressure which remained at about 210/130 on the same treatment.

7. Woman aged 45. Hypertension was discovered two years before the nephroangiography which showed a silent left kidney and multiple stenotic lesions of the right renal artery. A stenotic lesion of the aorta below the origin of the right renal artery was also revealed. In 1963 a left sided nephrectomy was performed without effect on the blood pressure.

Thus out of a group of seven hypertensive patients who had not responded satisfactorily to medical treatment and who were considered suitable for surgery three were found to derive benefit from operation. Of the five complications described following angiography none resulted in permanent disability.

2. *Cardioangiographies*

Amongst 46 cardioangiographies performed during the period 1963–1965 six cases with complications were found. These investigations were also performed by percutaneous transfemoral puncture.

Case report of complications following cardioangiography

1. (Earlier published by Boström et al. (4).) Woman aged 4 with a clinical diagnosis of aortic stenosis. Following catheterization and angiography the patient developed an occlusion of the right femoral artery which was successfully treated with streptokinase. This patient was taking oral contraceptive pills.

2. Man aged 46. Diagnosis: mild aortic incompetence. Following the angiography the patient developed a pulsating aneurysm in his groin which was successfully corrected surgically.

3. Man aged 38 with a diagnosis of aortic incompetence. Following the angiography the patient complained of a cold feeling of his right foot. There were no objective physical findings and the symptoms subsided rapidly.

4. Woman aged 43 with a presumtive diagnosis of aortic and mitral stenosis. Treatment with heparin and anticoagulants was begun following the finding of a thrombus in her left atrium at angiography. She developed a haematoma in the groin, but this vanished spontaneously.

5. Woman aged 51 with aortic and mitral stenosis. After the angiography the patient developed an abscess in her right groin. This was treated by drainage and antibiotics and the patient had no lasting disability. At admission this patient had slight splenic enlargement and a low platelet count. These findings subsided during the hospital stay.

6. Man aged 48. Diagnosis: myocardial tumour and tricuspid incompetence. Following angiography the patient had a cerebral embolus with a lasting hemianopia.

Table III. *Rate of complications at different investigations*

Kind of investigation	No. of cases	No. of complications
Nephroangiographies (all)	212	6 (2.8 %)
Nephroangiographies in the investigation of hypertension	101	5 (4.9 %)
Cardioangiographies	46	6 (13.0 %)
Coronary angiographies	71	3 (4.2 %)
Total	329	15 (4.6 %)

In this group of 46 patients 15 were operated. One died in connection with the operation. Six complications were observed and one case had lasting disability.

3. *Coronary Angiography*

Amongst the 71 coronary angiograms performed 1963–1965 three serious complications have been observed.

Case reports of complications following coronary angiography

1. Man aged 56. During the performance of coronary angiography the patient developed a pericardial haemorrhage which had to be drained. No open surgery was necessary, however, and the patient developed no further disabilities from this episode and it was found possible to repeat the investigation a fortnight later with no ensuing complications.

2. Woman aged 51. Following the coronary angiography the patient developed a left sided hemiplegia. She showed some improvement but remains hemiparetic.

3. (Earlier published by Boström et al. (4).) Woman aged 44. After the coronary angiography this patient developed an occlusion of her right femoral artery which was successfully treated with streptokinase. This patient was also taking contraceptive pills.

Four of these 71 patients were operated on. None died in connection with the operation. There were three serious complications, one with lasting disability.

Table IV. *Complications in 329 angiographies made by percutaneous arterial puncture*

	No. of cases
Bleeding at site of puncture	6
Infection at site of puncture	1
Occlusion of an artery in the leg	3
Cerebral accident	2
Allergic reaction	1
Hemopericardium	1
Spasm of an artery in the leg	1

SUMMARY OF THE RESULTS

In this group of 329 patients whose records were reviewed fifteen (4 6%) complications were found and their distribution is shown in Table III

In no case was the outcome fatal Two patients were left with permanent disability one with a hemianopia one with a moderate hemiparesis The different complications are listed in Table IV

DISCUSSION

In any diagnostic procedure which involves risks to the patient there must be a proper balance between the diagnostic gains and the number and severity of complications encountered We have limited our studies in this field to nephroangiograms which were performed in the work up of patients with hypertension and have only made a survey of cardioangiographies and coronary angiographies The latter have been discussed by McGuire (17) in a recent paper

The cause of the complications was often obscure but in some patients certain circumstances may be significant One of the patients who developed a haematoma at the site of the puncture had been given anticoagulant treatment and two of the three patients who developed an arterial occlusion in the lower limbs were taking oral contraceptives The third was found to have a slightly prolonged bleeding time on admission and had therefore been given vitamin K orally for one week The patient who developed circulatory shock following the investigation may have been hypersensitive to the injected contrast material as he had shown the same symptoms following pyelography two weeks earlier The complication with the pericardial haemorrhage is ascribed entirely to mechanical factors Finally the patient who developed an abscess at the puncture site had an enlarged spleen and a low platelet count before the investigation

Lang (15) has presented a survey of 11 402 percutaneous angiographies and described the most common complications This author found seven (0 06%) complications with fatal outcome 81 (0 7%) with serious complications and 325 (2 9%) with minor complications In Lang s investigation the most serious complications were found to be arterial thromboses or emboli as well as perforations of major blood vessels Amongst the minor complications intramural contrast injection and local haematomas are the most common Davidson et al (8) described 1000 aortograms and angiographies performed by arterial puncture in either arm or leg They found two deaths and fifty local reactions following these procedures They also found a higher incidence of complications following brachial artery punctures as compared with the femoral approach By a thorough study of the X ray films 31 cases were found to have some contrast injected subintimally Halpern et al (11) have likewise studied 1000 consecutive cases and found haematomas and bleedings in 1 7% and thrombosis and loss of pulse in the leg in 0 5 % They concluded that thrombosis is a more common complication in patients with cardiac disease and suggested that postexamination compression of the site of puncture is a dangerous routine in the investigation Aagaard et al (1) have studied 535 cases of arteriography and were unable to demonstrate any tendency to thrombosis but they found 29 haematomas and six complications with bleeding and one instance of embolisation Chamberlain et al (7) describe 107 aortographies with a complication rate of 12 4 % On the other hand Morris et al (20) claim to have performed 2500 aortograms without any serious complications

As a cause of the complications most authors agree that heart disease especially when associated with low output and arteriosclerotic disease is a predisposing factor Several authors including Lang (15) point out the value of a proper and meticulous technique including atraumatic puncture followed by careful compression after the examination In the present study no correlation between circulatory fitness and the rate of complications could be found yet it is noticeable that by far the highest percentage of complications occurred in connection with cardioangiographies It was impossible to estimate the technical difficulties in every single investigation retrospectively

Bergentz et al (3) stress the benefit of early detection and treatment of complications During a five year period these authors treated 51 complications out of an estimated number of 3000 arterial punctures per year In all patients with signs of lasting disability surgical treatment had been delayed Even in this series the complications were the ones usually encountered

thrombosis aneurysms or bleedings Bostrom et al (4) and Gripe (10) have also discussed the treatment of these complications

The value of nephroangiographies and the significance of renal artery stenosis in arterial hypertension have been widely discussed The investigation by Chamberlain et al (7) comprising 107 cases shows that 35 of these had pathological aortograms Fourteen of these were operated upon but only four with a good result and the nephroangiographies were also associated with a *complication rate of 12 4°* *Rees (20) described* 114 cases with pathological X ray findings in 35 (31 ′) of whom 9 (7 8°₀) were considered suitable for operation Two of these 9 died one in direct connection with the operation and in the remaining seven the blood pressure was unaffected In five of these patients biopsy of the contralateral kidney had been performed which showed changes of probable irreversible nature Kennedy et al (14) studied 750 cases of hypertension In 165 of these aortography was performed (145 through translumbar punction) Forty three patients (27%) showed signs of renal artery stenosis and in 27 (17%) this finding was considered to be the cause of hypertension Bunnell and Greene (6) have reported 127 cases in which they performed selective renal angiography They found renal artery stenosis in 32 cases of whom 12 were operated upon with good result in nine The complication rate in the investigations was found to be 6° This is a higher rate of detection than in other investigations In this connection Edling and Ovenfors (9) found microscopical evidence of renal damage in all of 18 dogs on which selective angiography had been performed Winter (24) claims that about 35° of the aortograms performed on hypertensive patients reveal renal artery or parenchymal disease which frequently is amenable to surgery

Indications for nephroangiographies in connection with hypertension have been put forward by McMichael (18) and Ask Upmark and Fagerberg (2) Ask Upmark and Fagerberg found ten positive nephroangiographies of 62 performed Two of these were operated on with good result and one with no effect on the blood pressure In our investigation a somewhat higher incidence of pathological nephroangiograms was found Twenty five in 101 cases were considered pathological and seven were selected for operation but only three derived any benefit In this group of 101 nephroangiograms five complications were found all of which must be considered potentially serious

The great optimism in connection with surgical treatment of renal artery stenosis following Goldblatt s experiment has to some extent changed into a more uncertain attitude Only 25–40 ₀ of the patients derive any benefit from an operation which is based on pathological nephroangiograms and it must be concluded that this finding in itself *cannot be a sufficient indication for operation* An interesting study was made by Holley et al (12) who describe post mortem angiograms in an unselected group of patients in order to study the frequency of renal artery stenosis between normo and hypertensive patients They describe 295 cases of which 39 during life had been *considered to be hypertensive In the group* of normotensive subjects stenosis was found in 49 ₀ and in the hypertensive group in 77°₀ They concluded that renal artery stenosis found on X ray examination in a group of patients over 50 years of age does not indicate a causal relationship to hypertension

Several authors have suggested contralateral renal biopsy to exclude irreversible changes The experience from this procedure seems as yet too limited to decide which changes might regress following the lowering of the blood pressure Others have suggested selective urine analyses by retrograde catheterization Unfortunately this method is not free from complications but may be of value in selected cases Kennedy et al (14) suggest that the combination of intravenous pyelography and the isotope renogram as a screening procedure gives an acceptable detection rate in association with safety and comfort

COMMENTS

The present study shows that nephroangiography as the primary method for detection of renal artery stenosis as a cause of hypertension gives relatively low dividends compared with the risks discomforts and costs Therefore it seems reasonable to perform other investigations in the first place which are safer but help to detect renovascular hypertension Such investigations could be e g The rapid sequence intravenous pyelogram described by Maxwell et al (16) the iso-

tope renogram advocated amongst others by Kennedy et al (14) and 'The angiotensin infusion test as described by Kaplan and Silah (13) If facilities are available an estimation of the renin like activity in the plasma as described by Brown et al (5) might be a good complement

If neither of these methods suggest a renovascular cause of hypertension a nephroangiography should not be performed

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HAEMOGLOBIN LEVEL AND RENAL FUNCTION IN PATIENTS WITH AND WITHOUT HYPERTENSION

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Abstract A comparison has been made between the haemoglobin level in normotensive and hypertensive patients with impaired renal function

Hypertensive patients have a considerably higher haemoglobin level than normotensive patients with the same degree of impairment of renal function.

Because of a higher daily production of creatinine the renal function assessed on the basis of the creatinine clearance is higher in hypertensive than in normotensive patients in relation to their serum creatinine concentration.

In cases of impaired renal function anaemia is such a well known and constant feature that it is generally supposed that renal function cannot be reduced to any appreciable extent without being accompanied by a certain—although varying—degree of anaemia

However in our out patients clinic for hypertensive patients we have on several occasions discovered that patients with increased serum creatinine values exhibited haemoglobin levels which were remarkably high as compared to the supposed impairment of renal function These findings gave incentive to a further study of the renal anaemia in our hypertensive patients By way of comparison a group of patients with renal insufficiency in the absence of hypertension is included

Reports have been published in the literature (1 2 3) indicating that the renal anaemia is less pronounced in hypertension than in other groups of patients with uraemia These findings which were confirmed by our studies will be discussed below

MATERIAL

The material is obtained partly from the patients admitted to the hospital during the period from January 1st 1965 to October 1st 1966 and partly from our out patients clinic for after treatment where in particular patients with hypertension and with chronic renal diseases are followed Patients with serum creatinine values equal to or above 1.3 mg per 100 ml in whom a reliable determination of creatinine clearance over three 24 hour periods was available were included (Patients with remarkably high serum creatinine values—i e higher than 6 0 mg per 100 ml—were not included since such values were found almost exclusively in patients with normal blood pressure and consequently such cases are not well suited for a comparison of the conditions in normotensive and hypertensive patients)

Furthermore we only included patients who as regards the renal function, were in a steady state Finally all patients were excluded in whom the haemoglobin level was supposed to be influenced by extra renal factors, such as bleeding recent blood transfusion, acute infection oedema etc

The material comprised 74 adult patients (35 normotensive and 39 hypertensive) All the normotensive patients had diastolic pressures equal to or lower than 100 mm Hg most of the readings ranging from 70 to 80 mm Hg All the hypertensive patients had diastolic blood pressures equal to or higher than 110 mm Hg, most of them considerably higher since these patients had severe hypertension requiring treatment (at the time of the examination some of the hypertensive patients had a more or less reduced blood pressure because of the treatment given as appears in Table III)

Seven of the 35 normotensive patients were males and 28 were females. The diagnoses were eight cases of chronic pyelonephritis 2 chronic interstitial nephritis (with a previous history of large intake of analgetics) one patient had disseminated lupus erythematosus and one hyperparathyroidism. Twenty six of the 39 hypertensive patients were males and 13 were females The diagnoses were 9 cases of essential arterial hypertension seven with chronic pyelonephritis (one of these was a diabetic) and three with chronic interstitial nephritis.

In the normotensive patients the average age was 60 6 years (males 9 3 years females 60 9 years) and in the hypertensive patients 51 7 years (males 53 7 years, females 47 9 years)

The haemoglobin determinations were carried out as

Table I *Renal function and haemoglobin values in normotensive and hypertensive patients*

	No	Age (y)	Serum creatinine (mg/100 ml)	Creatinine excretion (24 h/1 73 m²)	Creatinine clearance (ml/min/1 73 m²)	Haemoglobin (g/100 ml)
Normotensive						
Total	35	60 6	2 51	902	29 0	10 40
Males	7	59 3	2 58	1107	34 2	11 08
Females	28	60 9	2 49	850	27 6	10 [illegible]
Hypertensive						
Total	39	51 7	2 40	1275	4[illegible] 5	14 40
Males	26	53 7	2 37	1349	45 7	14 81
Females	13	47 9	2 48	1176	36 2	13 56

oxyhaemoglobin determinations employing a haemotest apparatus. Creatinine was determined according to Bonsness and Taussky's method (4) from this the total Jaffe positive chromogen substance was determined. The creatinine clearance values were consistently corrected to 1 73 sq.m. of body surface area Also the diurnal excretion of creatinine was corrected to 1 73 sq m of body surface area.

RESULTS

If the haemoglobin level and the serum creatinine value are compared directly a pronounced difference between the two groups is revealed (Table I) Whereas the average serum creatinine values are fairly identical in the two groups (normotensive patients 2 51 hypertensive patients 2 40 mg

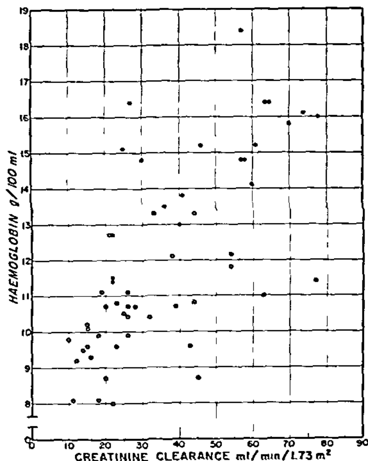


Fig 1 Creatinin values versus haemoglobin values in normotensive (○) and in hypertensive patients (●)

Table II *Individual data from 35 normotensive patients*

Case no	Sex	Age (y)	BP syst /diast	Serum creatinine (mg/100 ml)	Creatinine excretion (24 h/ 1 73 m)	Creatinine clearance (ml min/ 1 73 m²)	Haemoglobin (g/100 ml)	Diagnosis
1	♀	63	130/80	2 8	403	10	9 8	CIN
2	♀	75	130 90	4 7	744	11	8 1	CIN
3	♂	60	150/90	4 8	829	12	9 2	CIN
4	♀	62	120 80	3 2	645	14	9 5	LED
5	♀	77	140/80	5 2	1123	15	9 6	CIN
6	♀	75	120 80	2 0	432	15	10 2	CP
7	♀	74	165/85	2 8	605	15	10 1	DN
8	♀	79	170 65	2 9	668	16	9 3	CP
9	♀	83	150/80	1 8	467	18	9 9	CP
10	♀	48	140 80	3 0	778	18	8 1	CIN
11	♀	45	120/70	2 4	657	19	11 1	CIN
12	♀	41	165/100	3 0	864	20	10 7	CIN
13	♀	50	140/85	3 6	1037	20	8 7	CIN
14	♀	54	140/90	3 4	1028	21	12 7	CIN
15	♀	64	130 80	2 0	634	22	8 0	CIN
16	♀	47	110/75	2 4	795	23	10 8	CIN
17	♀	50	150 90	2 5	828	23	9 6	CIN
18	♀	40	130 80	3 2	1152	25	10 5	CP
19	♂	48	120 90	2 6	973	26	11 1	CIN
20	♀	57	140/80	2 6	973	26	10 4	CIN
21	♀	64	105/70	2 1	786	26	10 7	CIN
22	♀	64	105/70	2 0	748	26	9 9	CP
23	o	52	150/90	2 6	1048	28	10 7	CIN
24	♂	73	130 90	2 6	1198	3	10 4	CIN
25	♀	72	170/90	2 0	950	33	13 3	CIN
26	♀	43	135/80	1 8	985	38	12 1	CIN
27	♀	89	160/80	1 4	786	39	10 7	CP
28	♀	72	165/90	1 4	867	43	9 6	DN
29	♂	62	160/90	1 9	1204	44	13 3	DN
30	♂	60	140 90	2 1	1331	44	10 8	CIN
31	♀	50	140 80	1 7	1102	45	8 7	CP
32	o	60	140 80	1 5	1166	54	12 1	H
33	♀	50	150/85	1 5	1166	54	11 8	CIN
34	♀	64	150/90	1 3	1197	63	11 0	CP
35	♀	54	115/80	1 5	1663	77	11 4	CIN

CIN = chron interst nephritis LED = lupus erythem diss CP = chron pyelonephritis DN = diab nephropathy
H = hyperparathyr

per 100 ml) the average concentrations of haemoglobin are 10 40 g per 100 ml and 14 40 g per 100 ml respectively However it appears that when assessed on the basis of the creatinine clearance the renal function in the two groups differs with average values of 29 0 in the normotensive patients and 42 5 in the hypertensive

The reason for this is that the daily excretion of creatinine varies in the two groups In the normotensive group it is 902 mg per day and in the hypertensive group 1275 mg Naturally the sex distribution will influence the total creatinine excretion in the two groups and consequently we have stated the values for males and females separately in Table I It will be seen that in both sexes the creatinine excretion differs in normotensive and hypertensive patients This appears also from Table IV

In the following we shall deal with the relationship between haemoglobin values and renal function expressed by the creatinine clearance Since it is somewhat difficult to compare the materials directly we found it expedient to state our collective observations for the two groups These observations are given in Table II (normotensive) and in Table III (hypertensive)

The unevenness of the sex distribution has no particularly disturbing effect as regards the assessment of the difference between the normotensive and the hypertensive patients since the

Table III *Individual data from 39 hypertensive patients * in column 4 indicates that the patients were examined before the antihypertensive treatment was started Therefore the blood pressure of these patients (shown in column 5) indicates pre treatment values*

Case no	Sex	Age (y)	BP before treatment syst./diast.	BP during investigation syst./diast.	Serum creatinine (mg 100 ml)	Creatinine excretion (24 h/1 73 m)	Creatinine clearance (ml min 1 73 m²)	Haemoglobin (g 100 ml)	Diagnosis
36	♂	6[illegible]	210 140	170 130	5 2	1048	14	13 9	EH
37	♀	48	180 130	140 105	5.2	1[illegible]48	18	13 6	CIN
38	♂	58	[illegible]40 160	210 1[illegible]0	3 4	930	19	12.3	EH
39	♂	57		[illegible]00 110	4 0	1210	21	13 9	EH
40	♂	42	245/150	155/110	4 5	1361	21	15 4	EH
41	♀	44	230 150	200 110	2.5	992	2[illegible]	11 4	CP
42	♀	62	230 130	160 100	3 4	1077	2[illegible]	11 5	CIN
43	♀	60		200 110	3 0	930	22	12.7	CP
44	♀	36	[illegible]00 110	150 100	3 0	994	[illegible]3	11 7	DN
45	♀	50	[illegible]50 150	180 115	2.5	900	25	15 1	EH
46	♂	66	[illegible]50 140	170 115	2.5	97[illegible]	27	16 4	EH
47	♂	64	*	250 150	3 0	1296	30	14 8	EH
48	♂	57		180 110	3 2	14[illegible]8	31	11 8	EH
49	♂	69	190 1[illegible]0	170 110	2 6	1161	31	15 4	CP
50	♂	58	210 150	[illegible]00 170	2 0	950	33	12.9	EH
51	♂	58	[illegible]40 125	210 110	2 4	1210	35	10 8	EH
52	♀	44	190 120	160 90	[illegible]1	1058	35	15.2	CP
53	♂	52	210 150	170 110	2 7	1399	36	15 6	EH
54	♀	49	195 120	160 95	2.2	1140	36	13 5	CIN
55	♀	34		175 110	2.3	13[illegible]4	40	13 0	CP
56	♀	57	220 1[illegible]0	200 110	1 6	945	41	13 8	EH
57	♂	60		2[illegible]0 1[illegible]0	1 7	1126	46	15.2	EH
58	♂	57		230 130	2.0	138[illegible]	48	13 9	EH
59	♂	53	[illegible]30 150	115 80	2.1	157[illegible]	52	13 9	EH
60	♂	45	[illegible]40 150	140 80	1 9	1450	53	16 6	EH
61	♂	48		[illegible]0 150	1 8	14[illegible]6	55	14 4	EH
62	♂	50	200 150	180 110	1 7	1371	56	15 4	EH
63	♂	49	[illegible]0 130	160/90	2.1	17[illegible]4	57	14 8	EH
64	♂	52		195 1[illegible]0	1 4	1149	57	18 4	EH
65	♂	48		230 160	1 7	14[illegible]0	58	14 8	EH
66		4[illegible]	[illegible]40 145	[illegible]10 125	1 5	1274	59	16 1	EH
67	♂	52	[illegible]60 140	160 100	2.5	2160	60	14 1	EH
68		49	160 110	160 95	1 6	1401	61	15 [illegible]	CP
69	♂	6[illegible]	220 140	170 100	1 3	1198	64	16 4	EH
70	♂	5[illegible]		230 160	1 7	1567	64	16 4	EH
71		48	200 140	190 1[illegible]5	1 3	1236	66	13 5	EH
72	[illegible]	38		190 1[illegible]0	1 5	1312	70	15 8	EH
73	♂	48	170 110	140/90	1 3	1385	74	16 1	EH
74	♂	38		2[illegible]0 140	1 5	1685	78	16 0	EH

EH = ess hypertension CIN = chron interst. nephritis. CP = chron pyelonephritis. DN = diab nephropath

haemoglobin values within the two groups are almost identical in males and females This appears also from Table I

Conversely the renal function as such is markedly different in the two groups Nevertheless it appears directly that in the normotensive patients the haemoglobin level is lower than that found in hypertensive patients with the same degree of impairment of the renal function. This is illustrated in Fig 1

In order to substantiate this finding we compared the two groups in respect of a range of renal function with almost the same number of patients from the two groups This is the creatinine clearance range from 17 to 54 ml and the values are given in Table IV Whereas the average creatinine clearance values are almost identical (normotensive patients 30 6 ml, hypertensive patients 31 9 ml) the average haemoglobin concentration is 10 62 g per 100 ml in the normotensive patients and 13 76 g per 100 ml in the hypertensive group

Table IV *Renal function and haemoglobin values in a special group of normotensive and hypertensive patients (see text)*

	No	Age (y)	Serum creatinine (mg/100 ml)	Creatinine excretion (24 h/1 73 m²)	Creatinine clearance (ml/min/1 73 m)	Haemoglobin (g/100 ml)
Normotensive						
Total	25	57 6	2 28	941	30 6	10 6
Males	6	59 1	2 21	1154	38 0	11 40
Females	19	57 0	2 30	874	28 3	10 38
Hypertensive						
Total	24	53 3	2 74	1174	31 9	13 76
Males	14	56 8	2 71	1246	34 5	14 [illegible]0
Females	10	48 4	2 78	1073	[illegible]8 4	13 15

The same difference is found when males and females are evaluated separately

As stated in the section dealing with the patient material the great majority of the normotensive patients had chronic pyelonephritis or chronic interstitial nephritis whereas this type of patient was represented by the minority of the hypertensive group Therefore Table V presents a comparison between all the normotensive patients (group I) the ten hypertensive patients with chronic pyelonephritis or interstitial nephritis (group II) and the 29 hypertensive patients who belonged to the group of essential hypertension (group III) It will be seen that also in patient group II the haemoglobin level is higher than that found in the normotensive patients

Various conditions will now be dealt with which might be related to the difference in the haemoglobin level

Culture of clean voided urine showed growth in six of the hypertensive patients as against 18 of the normotensive subjects However it will be seen from Table VI that in the normotensive group the haemoglobin values found in patients with positive urine cultures (group I) do not differ greatly from those found in patients with sterile urine (group II)

In six of the hypertensive and in 23 of the normotensive patients there was a definite history of an extensive consumption of analgetics Furthermore we tried to estimate the number of normotensive patients who at the time of examination still used analgetics The possible influence on the haemoglobin values of this condition is shown in Table VII

Group I represents patients with previous and

Table V *Comparison of normotensive (I) and hypertensive patients with chronic pyelonephritis or interstitial nephritis (II) and cases diagnosed as essential hypertension (III)*

	Creatinine clearance (ml m n/1 73 m)	Haemoglobin (g/100 ml)
I	29 6	10 4
II	31 0	13 3
III	46 6	14 8

Table VI *Comparison of normotensive patients with urinary tract infection (I) and without (II)*

	Creatinine clearance (ml/min/1 73 m²)	Haemoglobin (g/100 ml)
I 18 pats	26 5	10 2
II 17 pats	31 6	10 6

Table VII *Normotensive patients and their use of analgetics*

I Former and present use II Former but not present use III No former or present use

	Creatinine clearance (ml/m n/1 73 m)	Haemoglobin (g/100 ml)
I 8 pats	28 8	10 8
II 15 pats	22 6	10 2
III 12 pats	3 [illegible] 5	10 4

Table VIII *Hypertension in relation to treatment with hypotensive drugs*

	Creatinine clearance (ml/min/1 73 m²)	Haemoglobin (g/100 ml)
18 pats under treatment	44 0	14 6
11 pats without treatment	50 7	15 0
Total	46 6	14 8

continued consumption of analgetics group II comprises patients with a previous consumption and group III represents patients without known use of analgetics No difference is found between the three groups

Finally it should be stated that some of the hypertensive patients were treated with hypotensive drugs at the time of examination All these patients received considerable doses of diuretics as a part of the treatment Table VIII presents the haemoglobin values in patients with essential hypertension with and without hypotensive treatment

DISCUSSION

As appears from our results we believe that there is a decisive difference between normotensive and hypertensive patients as regards the haemoglobin level in the presence of impaired renal function

This difference is particularly pronounced if only the level of the serum creatinine is taken into consideration However a certain increase *in the serum creatinine indicates a different* degree of impairment of renal function in the two groups This is caused by a systematic difference in the creatinine excretion and consequently in the production of creatinine in the two categories of patients

Enger and Blegen (9) pointed out that the daily excretion of creatinine was reduced in patients with renal disorders These authors were inclined to believe that this phenomenon resulted from a reduction in the muscular mass in patients with renal disorders

We found a higher daily excretion of creatinine in our patients with hypertension than in our patients with normotensive chronic renal diseases Most of our hypertensive patients were active persons working full time Conversely many of our normotensive patients were affected by their disorders to such an extent that they had to restrict their physical activities to a minimum Hence most likely our groups of patients too exhibit a difference in preserved muscular mass which might be of decisive importance

Hence in the light of the conditions outlined above a certain increase in serum creatinine must be evaluated differently in the two groups of patients This has had the effect that although our two groups are fairly identical as regards the level of the serum creatinine they differ in respect of the renal function assessed on the basis of the creatinine clearance

In the comparison of the two groups only a few hypertensive patients with severely impaired renal function are included No doubt this is due to the fact that from a prognostic point of view this condition is of such a serious nature that it will appear in our hypertension material on rare occasions only On the other hand the normotensive renal cases with slightly impaired renal function are also represented by a small number Because of the reduced production of creatinine *this category of patient will be found among* those with normal serum creatinine values i e below 1 3 mg per 100 ml

Also the difference in the sex distribution makes a direct comparison of our two principal groups difficult This difference is caused by the fact that the majority of the normotensive patients with chronic renal disorders are females whereas hypertensive patients with impaired renal function most often are males However the results *show that the haemoglobin level found in* the two groups is almost identical in males and females in relation to the co-existing impairment of renal function

As mentioned in the introduction patients with symptoms of acute urinary tract infection were excluded Nevertheless a chronic urinary tract infection might be an influential factor in the lower haemoglobin values found in the normotensive patients Culture of the urine was positive *more frequently in the normotensive than in the* hypertensive patients but as appears in Table VI a co-existing urinary tract infection does not seem to change the patients haemoglobin level

It is universally accepted that an extensive consumption of analgetics may give rise to impairment of the renal function but it might also directly influence the red cell formation resulting in anaemia. As would be expected a high consumption of analgetics was found most frequently in our normotensive patients However the results shown in Table VII contradict the presumption that this condition would be of decisive importance It appears from this table that the haemoglobin values in the patients who still used analgetics at the time of examination were not lower than in the remaining patients

The well known fact that impaired renal function results in anaemia has been investigated more systematically in a series of studies (5 8 14 15 16) In these studies the degree of the anaemia has been viewed most often in relation to the level of blood urea or to the serum creatinine Therefore a comparison with our results is difficult but an approximate estimate of the renal function on the basis of the values reported seems to indicate that the commonly accepted relationship between anaemia and renal function is almost in accordance with our findings in normotensive patients In the case of a renal function of 30 per cent of the normal haemoglobin values of about 12 g per 100 ml were found at 20 per cent values of 10 to 11 g per 100 ml and at 10 per cent 8 to 9 g per 100 ml

Therefore we suppose that the hypertensive patients in relation to their renal function present a higher haemoglobin level than that ordinarily found in the case of impaired renal function This theory is substantiated when the individual patients in the hypertensive group are taken into consideration Instances of a fairly pronounced impairment of renal function and at the same time completely normal haemoglobin values are encountered It has been considered whether long term massive hypotensive therapy can induce an increase in the haemoglobin level For the moment this problem is under investigation in a series of patients with hypertension and normal kidney function The preliminary results seem to indicate that after long term treatment with diuretics in large doses the haemoglobin level increases According to the results summarized in Table VIII this does not seem to happen in patients with renal insufficiency

As mentioned in the introduction previous authors have found conditions similar to our results Adams and Browns (1) for instance stated that the renal anaemia in hypertension was not so pronounced as that found in patients with glomerulonephritis

A report published by Ashe (2) contains numerous observations concerning anaemia and renal failure Although it is not stated directly the results show that hypertensive patients are less inclined to develop anaemia than other groups of patients if the renal function becomes impaired

Bock and Thedering (3) determined the circulating amount of erythrocytes in patients with mild and severe impairment of renal function Even at the time when the renal function was slighly impaired patients with chronic nephritis presented a decrease in the amount of erythrocytes In patients with malignant nephrosclerosis the volume of erythrocytes was increased and only when the impairment of renal function became severe did a decrease occur

As regards the cause of the difference in the haemoglobin level in our two principal groups it must naturally be borne in mind that the majority of our normotensive patients suffered from chronic pyelonephritis or interstitial nephritis whereas the hypertensive patients were mainly cases of essential hypertension It must of course be considered whether these two groups of diseases as such could result in differences in the haemoglobin level independent of the blood pressure However the results presented in Table V show that the hypertensive patients with chronic pyelonephritis or chronic interstitial nephritis also exhibit a relatively high haemoglobin level

It is an interesting but not clarified problem whether patients with hypertension have a higher haemoglobin level than the general population Volhard stated that the number of red blood cells will often be increased in patients with hypertension and the relationship between polycythaemia and hypertension described by Geisbock (10) is well known In more recent monographs relating to hypertension not much attention seems to have been paid to this problem However quite recently Christiansen et al (6) have again emphasized the frequent coincidence of polycythaemia and hypertension

The question has become of topical interest because of certain recent reports on a possible re

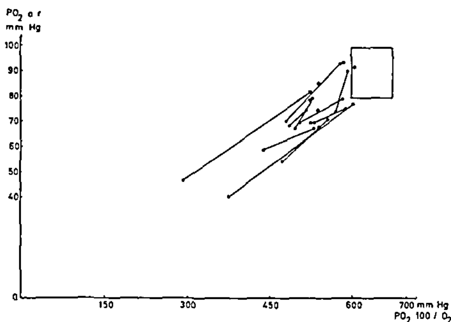


Fig 1 Arterial pO on air breathing and after oxygen breathing in patients with and without shock or pulmonary congestion O patients without shock • patients with shock.

in hospital demonstrated a marked increase in pO Only five patients however reached the normal range on air breathing and only one patient on oxygen breathing pCO in these patients varied between 28 and 45 mm Hg Most of the patients had slightly reduced pCO pH varied between 7 35 and 7 47

The pulmonary edema seen in some of these patients is demonstrated by Fig 2 showing marked pulmonary congestion on admission and roentgenological clearing of the lungs after four weeks

DISCUSSION

There are two possible explanations of this hypoxemia Firstly it might be due to venoarterial shunting in the lungs the blood bypassing pulmonary capillaries through totally unventilated parts of the lungs due to atelectasis or pulmonary edema Secondly the hypoxemia may be caused by uneven ventilation with disturbances of the ventilation perfusion ratio of the lungs In order to differentiate between these two conditions we have carried out calculations of the intrapulmonary shunt both on air breathing and



Fig 2 X ray of the chest in a patient with pulmonary edema and repeated X ray after four weeks demonstrating clearing of the lungs

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lationship between polycythaemia and renal arterial stenosis This problem was studied experimentally by Hansen (11) and in addition clinical reports of individual cases have been published (12 13) However Cotes and Lowe (7) showed that polycythaemia does not occur frequently in the clinic of renal arterial stenosis These authors found identical haemoglobin values in ten definite cases and in comparable control patients

Thirteen of our 29 patients with the diagnostic essential hypertension underwent renal angiography no evidence of renal arterial stenosis was found The reason why angiography was not carried out in the remaining 16 patients was that this examination was considered not to be indicated or to be contraindicated because of the impaired renal function It is impossible for us to decide whether any of these patients had renal arterial stenosis

It has been mentioned that our patients with hypertension generally had a higher daily production and excretion of creatinine than the normotensive patients We are inclined to relate this phenomenon to the fact that the general condition of our patients in the hypertensive group was usually better and they were more active It cannot be stated whether this would also influence their haemoglobin level

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THE CONCEPT OF SELF AS EXPERIENCED BY PATIENTS WITH A TRANSPLANTED KIDNEY

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Abstract The concept of Self' has both an immunological and a psychological meaning Fifteen patients on whom a kidney has been transplanted from related donors or from cadavers have been interviewed with regard to their psychological recognition of the kidney as part of their Self' The results are presented in a table and discussed

Table I *Patients and their reactions*

Pat. no	Sex	Age (y)	Donor	Post operative complications	Interval since operation	Present condition	Psychological reaction to transplant
1	o	22	Mother	Ureteric stenosis	1½ y	Moderate uremia	Rarely thinking of the kidney — completely accepted as his own
2	♂	33	Mother	Rejection	Short	Good	At the beginning a queer feeling later almost accepted
3	o	43	Mother	Rejection	14 mo	Good	Regard kidney as his own perhaps because it was his mother s and thus not alien
4	♂	17	Father	Repeated rejections	4 mo and 2½ y	Moderate uremia	Absolutely my organ —completely accepted
5	♂	17	Father	Repeated rejections	3/4 y	Transplant removed after 3 y Deceased	An alien thing —never accepted the kidney as his own
6	♀	34	Father	Liver damage	6 mo	Good	At first a queer feeling having someone else s kidney but now considers the kidney to be her own
7	o	40	Sister	Hepatitis	Short	Good	Why think about the kidney? Completely accepted it as his own
8	o	30	Brother	Rejection	6 mo	Good	Considers the kidney to be his own
9	♂	47	Brother	Rejection Fistula	½ y	Deceased	Considered the kidney alien at first but later he accepted it as his own
10	♂	34	Sister in law	Rejection Liver damage	3 mo	Good	"Regards kidney as his own no feeling of a foreign body
11	o	40	Free kidney	Fistula	1½ y	Good	No problem having someone else s kidney Completely accepted as his own Advantage not to know from whom
12	♂	37	Cadaver	None	2 mo	Deceased	Annoyed by the transplant, but regarded it as his own
13	o	41	Cadaver	Rejection	2 mo	Good	"Felt like being pregnant with someone else s kidney at first. Gradually accepted the kidney as his own
14	o	46	Cadaver	Stomatitis	Short	Good	No adverse sensations Completely accepted the kidney as his own Does not want to know who the donor was
15	o	58	Cadaver	Diabetes	2 y	Good	No adverse sensations Completely accepted the kidney as his own Does not want to know who the donor was

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THE CAUSE OF ARTERIAL HYPOXEMIA IN ACUTE MYOCARDIAL INFARCTION

O Storstein and K Rasmussen

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Abstract The mechanism of arterial hypoxemia commonly found in patients with acute myocardial infarction has been studied in 24 patients with polarographic determination of arterial pO_2 before and after ten minutes of pure oxygen breathing

Most of the patients have a reduction in arterial pO on air breathing and do not attain normal values for pO on oxygen breathing Patients in shock or with pulmonary congestion show more severely reduced values of pO both on air breathing and on oxygen breathing Calculation of the so-called physiological shunt on air breathing and the so called anatomical shunt on oxygen breathing demonstrates that most of the reduction in arterial pO is due to disturbance of the ventilation perfusion relationships in the lungs, but a considerable amount of the reduction in pO is caused by shunting of pulmonary arterial blood through non ventilated parts of the lungs

Polarographic determinations of arterial oxygen tension (pO) in patients with acute myocardial infarction of recent years have demonstrated a reduced arterial pO especially in patients with shock (6) and with pulmonary congestion (1 7) Studies by Valentine et al (13) have indicated that disturbances of the ventilation perfusion relationship in the lungs is responsible for the reduced arterial pO The reduction might alternatively be explained by shunting of blood through nonventilated parts of the lungs

MATERIAL AND METHODS

Twenty four patients with acute myocardial infarction were studied at Medical Department B University Hospital Rikshospitalet Oslo Determinations of arterial pO pCO and pH for all patients were done on admission. In patients with signs of shock or pulmonary congestion repeated studies were made about four weeks later Arterial blood was analyzed with a polarographic electrode (Eschweiler Kiel) Arterial pCO was measured with the same instrument and pH with the Astrup apparatus The patients in supine position were given pure oxygen to breathe for ten minutes from a tank through a bag with mouthpiece attached and arterial blood samples were collected for repeated analyses of blood gases

Calculations

Calculation of the shunt on air breathing the so-called physiological shunt has been carried out according to the usual formula

$$\frac{Qs}{Q} = \frac{Cc_{O} - Ca_{O}}{Cc_{O} - C\bar{v}_{O}}$$

The tables of Dill were read to find the saturation that blood would have if equilibrated with mean alveolar air The arteriovenous oxygen difference has been assumed to be 40 ml/l The shunt on oxygen breathing the so called anatomical shunt has been calculated using the same equation and under the assumption that all nitrogen has been washed out from the alveoli during the period of oxygen breathing The same assumed arteriovenous oxygen difference has been used in this calculation 40 ml/l

Studies on normal individuals in our laboratory have demonstrated arterial pO between 80 and 100 mm Hg on air breathing and between 600 and 680 mm Hg on oxygen breathing

RESULTS

As demonstrated in Fig 1 most of the patients had reduced arterial pO on admission Only six patients were in the normal range on air breathing and only four on oxygen breathing Most of the patients had a slight reduction of arterial pO to about 70 mm Hg on air breathing and between 500 and 600 mm Hg on oxygen breathing Patients in shock or with marked pulmonary congestion on admission had a more severely reduced arterial pO down to 40 mm Hg on air breathing and to 300 mm Hg on oxygen breathing

Repeated studies in patients with signs of shock or pulmonary congestion during their stay

The fundamental biological and philosophical problem of differentiation between self and not self has been analyzed in our time by investigators from the two cultures—by Burnet and Fenner (2) from the immunologists point of view and by Huxley (4) from the psychedelics Using medicine as a bridge between them it occurred to us that kidney transplantation might offer an experimental platform not only for acceptance and rejection but also for the concept of self (1) For this reason we have invited a number of patients who have received a kidney transplant in the Department of Surgery at Serafimerlasarettet (3) to offer their opinions thoughts and feelings about their kidney transplant —whether they have accepted it in a wider sense than that of immunology proper

As seen from Table I the great majority of these patients have immediately or gradually accepted the kidney as their own Only in one case—the only one in which the transplant had to be removed after three years—could the patient not accept the kidney as being his own Three patients with cadaver or free kidneys are positively disinterested in knowing about the donor while anoth r one believes the fact that he has got his mothers kidney to facilitate acceptance It may be however that an anonymous kidney is most easily accepted and that reaction to kidneys from near relatives is conditioned to some extent by feelings of affection or hostility towards the particular donor

In summary these patients seem to have a remarkable capacity for psychological acceptance of a foreign organ incorporated in their body This is in a way the opposite of the feeling of some cardiac patients who may experience their own heart when suffering from arrhythmia as someone else s heart

From some of the expressions used by our informants it cannot however be excluded that the acceptance is brought about through a denial or suppression of a subconscious fe ling which might be harmful if it came to the surface

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From Medical Department B University Hospital Rikshospitalet Oslo Norway

Abstract The mechanism of arterial hypoxemia commonly found in patients with acute myocardial infarction has been studied in 24 patients with polarographic determination of arterial pO before and after ten minutes of pure oxygen breathing

Most of the patients have a reduction in arterial pO on air breathing and do not attain normal values for pO on oxygen breathing Patients in shock or with pulmonary congestion show more severely reduced values of pO both on air breathing and on oxygen breathing Calculation of the so-called physiological shunt on air breathing and the so-called anatomical shunt on oxygen breathing demonstrates that most of the reduction in arterial pO is due to disturbance of the ventilation perfusion relationships in the lungs but a considerable amount of the reduction in pO is caused by shunting of pulmonary arterial blood through non ventilated parts of the lungs

Polarographic determinations of arterial oxygen tension (pO) in patients with acute myocardial infarction of recent years have demonstrated a reduced arterial pO especially in patients with shock (6) and with pulmonary congestion (1 7) Studies by Valentine et al (13) have indicated that disturbances of the ventilation perfusion relationship in the lungs is responsible for the reduced arterial pO The reduction might alternatively be explained by shunting of blood through nonventilated parts of the lungs

MATERIAL AND METHODS

Twenty four patients with acute myocardial infarction were studied at Medical Department B University Hospital Rikshospitalet Oslo Determinations of arterial pO pCO and pH for all patients were done on admission. In patients with signs of shock or pulmonary congestion repeated studies were made about four weeks later Arterial blood was analyzed with a polarographic electrode (Eschweiler Kiel) Arterial pCO was measured with the same instrument and pH with the Astrup apparatus The patients in supine position were given pure oxygen to breathe for ten minutes from a tank through a bag with mouthpiece attached and arterial blood samples were collected for repeated analyses of blood gases

Calculations

Calculation of the shunt on air breathing the so-called physiological shunt has been carried out according to the usual formula

$$\frac{Qs}{Q} = \frac{Cc\,o - Cao}{Cc\,o - C\bar{v}\,o_2}$$

The tables of Dill were read to find the saturation that blood would have if equilibrated with mean alveolar air The arteriovenous oxygen difference has been assumed to be 40 ml/l The shunt on oxygen breathing the so-called anatomical shunt has been calculated using the same equation and under the assumption that all nitrogen has been washed out from the alveoli during the period of oxygen breathing The same assumed arteriovenous oxygen difference has been used in this calculation 40 ml l

Studies on normal individuals in our laboratory have demonstrated arterial pO between 80 and 100 mm Hg on air breathing and between 600 and 680 mm Hg on oxygen breathing

RESULTS

As demonstrated in Fig 1 most of the patients had reduced arterial pO on admission Only six patients were in the normal range on air breathing and only four on oxygen breathing Most of the patients had a slight reduction of arterial pO to about 70 mm Hg on air breathing and between 500 and 600 mm Hg on oxygen breathing Patients in shock or with marked pulmonary congestion on admission had a more severely reduced arterial pO down to 40 mm Hg on air breathing and to 300 mm Hg on oxygen breathing

Repeated studies in patients with signs of shock or pulmonary congestion during their stay

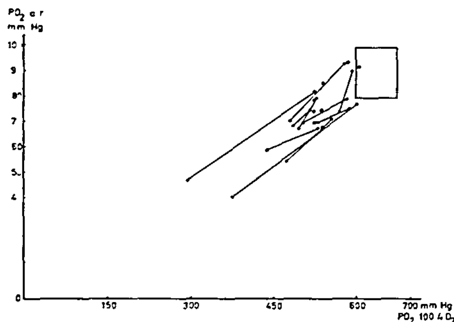


Fig 1 Arterial pO on air breathing and after oxygen breathing in patients with and without shock or pulmonary congestion. O patients without shock ● patients with shock.

in hospital demonstrated a marked increase in pO Only five patients however reached the normal range on air breathing and only one patient on oxygen breathing pCO in these patients varied between 28 and 45 mm Hg Most of the patients had slightly reduced pCO pH varied between 7 35 and 7 47

The pulmonary edema seen in some of these patients is demonstrated by Fig 2 showing marked pulmonary congestion on admission and roentgenological clearing of the lungs after four weeks

DISCUSSION

There are two possible explanations of this hypoxemia Firstly it might be due to venoarterial shunting in the lungs the blood bypassing pulmonary capillaries through totally unventilated parts of the lungs due to atelectasis or pulmonary edema Secondly the hypoxemia may be caused by uneven ventilation with disturbances of the ventilation perfusion ratio of the lungs In order to differentiate between these two conditions we have carried out calculations of the intrapulmonary shunt both on air breathing and



Fig 2 X-ray of the chest in a patient with pulmonary edema and repeated X ray after four weeks demonstrating clearing of the lungs

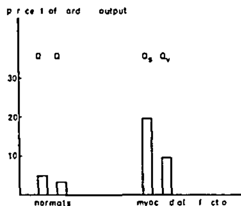


Fig 3 Mean values of calculated shunt on air breathing (Q) and on oxygen breathing (Q) in normal individuals and in patients with acute myocardial infarction

on oxygen breathing Calculation of the shunt on air breathing (Fig 3) demonstrates a so-called physiological shunt varying from 5 to 44% (mean 18) On oxygen breathing the calculated so called anatomical shunt varies from 3 to 23 % (mean 9) The difference between these two values is an expression of the amount of uneven ventilation perfusion of the lungs Uneven distribution of the ventilation perfusion relationship in the lungs requires the presence of a third gas such as nitrogen in the hypoventilated alveoli to cause a significant alveolar arterial oxygen gradient When 100 oxygen is breath d no such third gas is present There is only oxygen water vapor and CO in the alveoli A persisting alveolar arterial pO gradient is then caused by the veno-arterial shunt (4)

Thus *most of the calculated shunt on air* breathing is due to disturbances of the ventilation perfusion relationship but on oxygen breathing there is still left a shunt of 9 which must be due to pulmonary arterial blood bypassing ventilated parts of the lungs Our assumption of an arteriovenous oxygen difference of 40 ml/l may lead to an overestimation of the shunt If the arteriovenous oxygen difference is reduced to 60 ml/l calculation of the anatomical shunt would show a mean value of 7 instead of 9 (5)

Similar values for the anatomical shunt were found by Buschmann et al (1)—a mean value of 7 6 for Q_{VA} during oxygen breathing in 18 patients with acute myocardial infarction In their calculation they used an arteriovenous oxygen difference of 64 ml/l

Further investigation of the true right to-left shunt may be done by radioactive methods as shown by Mellemgaard (8) We have tried to estimate the shunt by the use of ^{85}Kr (3) but we like Mellemgaard et al (9) found that this method is not sufficiently accurate

The ultimate cause of the reduced respiratory function in acute myocardial infarction must be sought in left ventricular failure with pulmonary congestion As mentioned the most pronounced hypoxemia was found in patients in shock or with clinical signs of pulmonary congestion In Buschman et al s study (1) this is illustrated by a positive correlation between arterial pO and stroke volume in patients with acute myocardial infarction

From a practical viewpoint in the management of patients with acute myocardial infarction we feel that it is important to point out that most of these patients have a low arterial pO and that most of the patients show a considerable rise in pO on oxygen breathing Even patients in shock show a rise in arterial pO up to about 300 mm Hg that is more than three times normal values on air breathing We feel that it is important to stress the benefit of oxygen breathing in acute myocardial infarction The benefit may be derived in two ways in the first place by relieving the myocardial ischemia in the peripheral zones of myocardial infarction by hyperoxygenation of coronary arterial blood reaching the infarcted area through collaterals (10) Secondly it may benefit the general status of these *patients to relieve tissue anoxia and in this way* contribute to a rise in arterial pressure by increasing peripheral resistance (11) There is usually a fall in cardiac output due to a fall both in heart rate and stroke volume (1 2) Thus the work of the left ventricle may be unchanged or fall depending on the magnitude of changes in blood pressure and cardiac output Measurement of pulmonary arterial pressure during oxygen breathing has not be n carried out in these patients One should expect it to fall as it commonly does in patients with h art failure (12) In this way there will be a fall in the work of the right ventricle Further studies of the hemodynamic effect of oxygen breathing are required to clarify its effect on the general and regional circulation

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RENAL URATE DEPOSITS IN CHRONIC RENAL INSUFFICIENCY

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Abstract Report of two cases of hyperuricaemia secondary to chronic renal insufficiency with tophi in the kidneys Actual hyperuricaemic factors in connection with long uraemic conditions are discussed. The risk of secondary hyperuricaemia accentuating the renal damage is stressed

Chronic uraemia with secondary hyperuricaemia is rarely followed by gout but gouty kidneys are the most serious complication of primary hyperuricaemia and gout and often result in uraemia (18) With the improved methods of treatment of chronic renal insufficiency e g dialysis hyperuricaemia has become more important because of the increased duration of survival of the patients and the increased risk of injury by urate deposits (5) Now gouty kidneys seem to be found more often at autopsy of subjects without a known history of gout (12) We have recently seen two such cases which are described below

CASE REPORTS

Case 1

A man born in 1901 No known heredity for gout or joint symptoms Previously felt well In 1947 he had an infection of the upper respiratory tract followed by acute glomerulonephritis after which renal function was moderately impaired In 1954 he had arterial hypertension which responded favourably to treatment with antihypertensive drugs which were afterwards withdrawn In 1963 the patient had an attack of high grade fever which lasted for one week Two months later he sought medical advice because of fatigue Examination revealed B P 00/130 mm Hg ESR 137 mm/h normal liver function tests Hb 6 4 g 100 ml Sternal punctate reactive bone marrow with a certain degree of haemolysis Treatment with Salures K® was started In 1964 he still had anaemia and the ESR was still increased In 1965 he complained of diffuse pain in the right half of the abdomen In May 1966 the patient fell ill with right sided abdominal pain which was soon followed by jaundice The urine became dark ESR 106 mm h reticulocytes 10 of erythrocytes serum haptoglobin 0 mg/100 ml Coombs test direct and indirect positive urine output 1200 ml/24 h N P N 24 mg/100 ml endogenous creatinine clearance 58 ml/min Sternal punctate shift to the left of the normoblastic cells with signs of mild haemolysis In August 1966 the patient was still jaundiced and the discovery of immunisation with irregular antibodies explained the haemolysis

Laboratory studies showed B P 200/100 mm Hg, Hb 7 2 g/100 ml urine output 700–800 ml/24 h endogenous creatinine clearance 31 ml/min N P N 91 mg/100 ml and serum uric acid 22 mg/100 ml Obstructive jaundice developed and on August 25 choledocholithotomy was done The postoperative course during which the jaundice persisted was complicated by anuria fever and shock During the latter half of August the patient received all to ether 24 bottles of blood Haemodialysis was started on August 27 but three days later the patient died.

Autopsy

Gross examination The kidneys were somewhat large (total weight 310 g) but of normal shape The surface was coarsely granular and fairly pale The cut surface showed a reduced cortex and a reduced medullary zone and abundant peripelvic fat The medulla contained yellowish crystal like deposits The renal pelvis and the ureteric mucosa were of normal appearance

Microscopic examination Pieces of the kidney and of most other organs were fixed in 10 neutral formalin embedded in paraffin and stained with haematoxylin and eosin as well as according to van Gieson and McManus

The narrow cortex contained a number of hyaline spheres but most of the glomeruli were preserved with slight hyalinisation of the walls of the afferent and efferent arterioles. Bowman's capsules was somewhat dilated and showed moderate pericapsular fibrosis The proximal tubules exhibited the picture of osmotic nephrosis The distal convoluted tubules and the collecting tubules contained hyaline and pigment casts The walls of the smaller arteries were fibrously thickened and the arterioles showed moderate hyaline thickening of their walls The medulla showed moderate homogeneous interstitial fibrosis poor in cells The subcortical layer and the intermediate zone of the medulla contained numerous different-sized granuloma like formations (Fig. 1 *a* and *b*) built up by peripheral condensation of loose connective tissue containing polymorphonuclear leukocytes and some lymphocytes which increased in number towards the

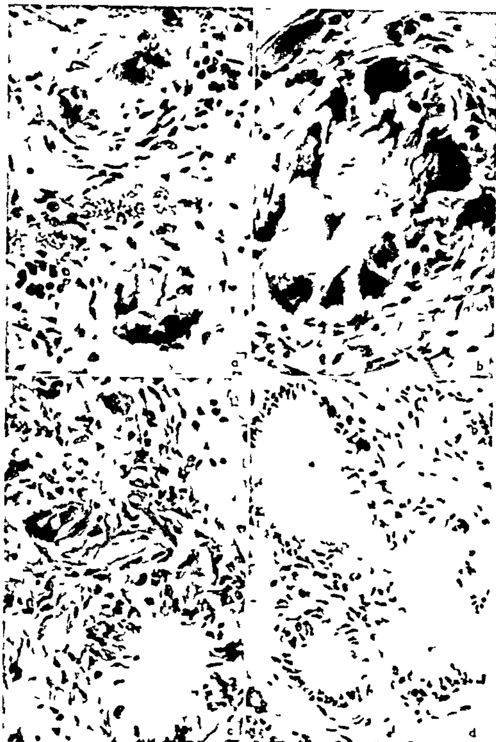


Fig 1 (*a*) Two collecting tubules in the intermediate zone of the medulla with gradual destruction and necrosis of the epithelium and adjacent connective tissue intraluminal foreign body giant cells and scattered lymphocytes and macrophages in the interstitial connective tissue Haematoxylin–eosin 0

(*b*) Damaged collecting tubule in the subcortical zone with extensive giant cell reaction around hollows after dissolved urate crystals in the preparation, and incipient interstitial fibrosis. Haematoxylin–eosin 370

(*c*) Remains of two collecting tubules in the intermediate zone of the medulla the upper intracellular urate crystals in giant cells the lower peripheral star shaped granuloma with macrophages giant cells and intercellular oval spaces and central amorphous material. McManus stain 370

(*d*) Tophus in intermediate zone of medulla with extensive interstitial fibrosis and incipient destruction of adjacent collecting tubules Haematoxylin–eosin 95

centre occup ed by a stellate arrangement of macrophages and foreign body giant cells and needle shaped lum na In some of the granulomas the centre consisted of a more or less homogeneous acidophilic mass while in others the picture was dominated by spaces and giant cells and in some by polymorphonu lear leukocytes and s attered giant cells Some of the collecting tubules showed signs of incipient destru tion of the epithelium and a varying rumber of polymorphonu lear cells as well as a few macrophages and giant cells

The examination thus showed gouty kidneys nephrosclerosis osmotic nephrosis and shock kidneys The examination of the other organs revealed signs of the following conditions haemo lytic anaemia (according to case records) with enlargement of the liver (wt 2350 g) and spleen (wt 1400 g) with multiple infarcts of the spl en (wt 550 g) uraemic pericarditis and circulatory insufficiency with pulmonary oed ma a thrombus in the left external iliac vein and status after tracheotomy and choledocholithotomy

Case 2

A man born in 19?3 No known heredity for gout or joint symptoms Previously felt well. In 1959 the patient had an infection of the upper respiratory tract followed by haematuria In 1960 the systolic blood pressure was 40 mm H X ray examination of the kidneys showed nothing remarkable In 1961 treatment with saluretics (th azide preparation) was started In 1962 the patient had occas onal mild pain of the left wrist In 1963 the wrist suddenly became very painful and swollen and the patient sought med cal advice Repeated roentgen exa mination of the wrist revealed nothing of interest The symptoms responded favourably to oral treatment with Butazol d n The laboratory values noted at that time were as follows ESR 83 mm/h B P 00/120 mm Hg, AST pos proteinuria N P N 32–46 mg/100 ml Con centration test spec fic gravity of urine 1 016 Fundus hypertonicus grade III Roentgen examination showed kidneys to be somewhat small The patient was sent hom with a prescription for Hygroton® In 1964–65 the pa t ent occasionally had pain in various jo nts The ESR was constantly elevated the electrophoret c pattern of the serum was normal The AST was positi e and the ASTA negati e The serum uric acid was increased and treatment with Probenecid was started In March 196(the patient still had proteinuria ESR 66 mm/h Hb 11 ? g 100 ml serum uric acid 15 m 100 ml B P _ 0 1_5 mm Hg plasma creatinine 6 7 mg 100 ml and creat n ne clearance 1 ml min i osthenuria Hygroton® and Probenecid were withdrawn because renal function was so poor The patient was sent home on Aldomet® and I mel n® but in September th t year he was re adm tted this t me to the emergency un t On adm ssion Hb 6 9 g 100 ml plasma creatinine ? mg 100 ml B P 190 1 0 mm Hg ESR 109 mm h ur ne output 180–400 ml 4 h Treatment with d alysi was conside ed but dur ng the investigation the patient developed uraemic pul monary oedema pericarditis acidosis and anuria Peri toneal d alysis was started but peritonitis supervened and the patient died in October 1966

Autopsy

Gross examination The kidneys were of normal shape but small (total wt 180 g) their surface was finely nodular with elevated punctate circumscribed areas Their colour varied between pale gray and dark red The cut surface showed the medullary zone to be hyperaemic and the cortex strikingly thin The amount of peripelvic fat was increased The medulla contained numerous yellow green long crystal like deposits lying in the same d rec tion as the collecting tubules The renal pelvis and the ureteric mucosa were of normal appearance

Microscopic exa ninatioi Pieces of the kidneys and of most other organs were fixed in 10% neutral formalin embedded in paraffin and stained with haematoxyl n and eos n as well as accord ng to van Gieson Ladewig *Perdrau and M Manus*

The normal architecture of the kidneys was no longer recognisable the cortex was severely reduced and there were only a few fairly well preserved glomeruli Most of the glomeruli consisted of hyaline spheres with adhesion between the parietal and visceral layer of Bowman s capsules The renal tubules were severely atroph ed esp cially in those areas where the glomeruli had been destroyed The distal convoluted tubules contained numerous hyaline casts The interstitial connective tissue was increased in amount and contained some bands and scanty focal clusters of lymphocytes The walls of larger and smaller arteries were markedly thickened and the lumina of some arterioles were almost completely obliterated The medulla showed moderate congestion of the blood and a number of collecting tubules were dilated and contained hyaline casts The subcortical layer and the intermediate one of the medulla showed several large and small formations consisting of per pheral con nective ti sue zones parts of which were fairly broad and dense These zones enclosed an area with macrophages and foreign body giant cells as well as a few polymorphonuclear leucocytes and lymphocytes Centrally a homogeneous partly foamy mass was split up in som parts by needle shaped spaces (Fig 1 *c* and *d*) In other areas of the medulla the picture was rem niscent of a granuloma with a preponderance of giant cells and stellate arrangement of needle shaped spaces wh le in further areas the polymorphonuclear leucocytes were preponderant Some collect ng tubules sl owed signs of incip ent destruction of the epithelium g ant cells macro phages and lymphocytes

The examination thus showed chronic glomerulonephritis and gouty kidneys The examination of the other organs revealed signs of the follow ing conditions uraemic pericarditis and pleurisy signs of hypertension with hypertrophy of the left half of the heart (wt 640 g) signs of cir culatory insufficiency with general dilatation of

the heart pulmonary oedema acute and chronic congestion in the liver (wt 2000 g) and spleen (wt 290 g)

DISCUSSION

Primary gout is often followed by renal disease ushered in by proteinuria and occasionally also by haematuria Uraemia sometimes develops Urate deposits in the renal parenchyma with a surrounding giant cell reaction and later formation of tophi are pathognomonic of gouty kidneys The lesions finally result in the development of contracted kidneys Most cases also show vascular injuries of the type seen in nephrosclerosis and pyelonephritis (15) In histological sections fixed in formalin the urate crystals are dissolved and leave behind hollow spaces Two types of such deposits have been described viz star shaped deposits in the collecting tubules with destruction *of the epithelium and secondly feather shaped* deposits in the interstitial tissue of the pyramids The latter are said to be typical of primary gout (1 12)

Secondary hyperuricaemia is liable to occur in a variety of diseases with an excessive nucleo protein turnover and extensive tissue destruction such as in myeloid leukaemia polycythaemia rubra vera and haemolytic anaemia (17) Clinically manifest gout is a more common accompaniment of primary hyperuricaemia than of secondary hyperuricaemia despite the higher concentration of the uric acid in the blood in the latter condition In primary gout there is often a lack of a uric acid binding α_1- globulin and the *presence of this globulin in secondary hyper* uricaemia increases the uric acid binding capacity of the blood and suppresses the tendency to deposition of monosodium urate (2)

Secondary hyperuricaemia occurs in uraemia but rarely long enough to give rise to gout (14) Mayne reported 27 patients with gout including five with histologically verified glomerulonephritis (11) Four of these five had urate deposits in the kidneys but only two of them showed signs of renal disease before the onset of the joint symptoms Talbott and Terplan found renal insufficiency in 18 of one autopsy series of 191 subjects with gout and in 25% of another consisting of 89 such subjects (15) The entire material included only one subject with signs of acute glomerulonephritis

Nephropathy due to lead poisoning can cause gout (8) During treatment of renal insufficiency with dialysis diffuse goutlike symptoms may develop and urate deposits have been found in the tissues (5 10) The possible effect of hyper uricaemia on the further course of the renal disease in such patients is still obscure

The formation of tophi depends to a large extent on the local environments in the tissue Locally leukocytes supply lactic acid and lower the pH which favours the deposition of urates (10) In patients with uraemia the local inflammatory response is weak (13) In such patients the tissue reaction to sodium urate crystals is also suppressed possibly because of a change in the solubility of urate in uraemic conditions (4) Probenecid increases the excretion of uric acid in the proximal tubules In renal disease this mechanism may fail The risk of deposition of *urates in the kidneys is greater if the pH of the* urine falls below 6 0 especially if the concentration of the uric acid is increased as it is during treatment with Probenecid (9)

In some patients treatment with thiazide can cause hyperuricaemia by its effect on the tubular transport of uric acid (7) and if they develop gout they probably had latent gout before treatment (3)

Hyperuricaemia in association with hypertension occurs in about half of the cases In untreated hypertension with normal N P N the concentration of the uric acid in the serum is increased in about one fourth of all cases In the treatment of hypertension with thiazide preparations or some other antihypertensive drugs the number of cases with hyperuricaemia increases considerably and of those in whom the N P N increases about two thirds develop hyperuricaemia The combination of hypertension low glomerular filtration and decreased uric acid clearance is common though a relatively normal glomerular filtration is often seen in association with decreased uric acid clearance This is interpreted as a sign of a changed tubular transport of uric acid in hypertension probably decreased tubular secretion (6)

In the cases described here the haemolysis in case 1 probably was the primary cause of a slight hyperuricaemia The kidneys showed no sequelae after previous glomerulonephritis The hypertension may have injured the kidneys as reflected

by the moderate impairment of renal function and this together with thiazide therapy might have caused fairly early hyperuricaemia too The successively increased haemolysis resulted in high concentration of the uric acid in the blood the excretion in the tubules increased and urates were deposited in the kidneys which in turn accentuated the renal damage Finally there occurred a renal lesion that resulted in hypoxia of the kidney with consequent cessation of renal function The scanty fibrosis around the large intratubular urate deposits indicated that the latter were of recent date an observation fitting in with the fact that the patient had had a pronounced renal insufficency for about half a year Microscopical examination revealed no feather shaped deposits The patient had no known heredity for gout or joint symptoms and thus nothing argued for primary hyperuricaemia

In case 2 the chronic glomerulonephritis had impaired renal function for a long time and this was the main cause of the patient s hyperuricaemia Hypertension might have contributed to the hyperuricaemia and the patient might have been unusually sensitive to thiazide The prolonged hyperuricaemia resulted in relatively early depositions of urates in the kidneys for histologically the tophi were surrounded by marked fibrosis Medication with Probenecid may have accentuated the deposition of the urate especially in the nephrons that had retained their capacity to acidify the urine The extensive formation of tophi also accelerated the destruction of the kidney The absence of heredity for gout and of feather shaped interstitial deposits of urates argues against the hyperuricaemia being primary The atypical joint symptoms might have been symptoms of gout secondary to uraemia

It is probable that uric acid precipitates (urates) will in future be a common finding at autopsy of subjects who have had renal insufficiency because patients with uraemia survive longer than before and secondly because thiazide preparations are now widely used Urate deposits in the kidneys probably accelerate the destruction of these organs Allopurinol a xanthine oxidase inhibitor suppresses the production of uric acid irrespective of the functional state of the kidneys and will probably therefore find considerable use in the treatment of hyperuricaemia in association with renal damage (16)

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PYELONEPHRITIS LIKE LESIONS AS A LATE EFFECT OF DIFFUSE INTRAVASCULAR COAGULATION

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Abstract A short episode of diffuse intravascular coagulation caused a range of severe kidney alterations in rabbits which did not die immediately with renal cortical necrosis Histology resembled that of chronic pyelonephritis The rabbits exhibited a steep but completely reversible rise in blood creatinine level and ultimately no abnormalities of the urine Blood pressure remained normal It is supposed that comparable situations may arise in human pathology

In recent years diffuse intravascular coagulation has been recognized as a complicating factor in many clinical conditions for example in most acquired coagulation disorders of pregnancy certain types of sepsis excessive tissue damage severe haemolysis some disseminated malignancies and in Gasser s syndrome (8 13)

In severe diffuse intravascular coagulation in man as in the generalized Sanarelli Shwartzman phenomenon in animals renal cortical necrosis may occur In less severe cases in man temporary impairment of renal function as measured by serum creatinine values has been observed (9) Very little is known about the histological renal damage resulting from these milder forms of diffuse intravascular coagulation and we therefore carried out a series of experiments in rabbits

MATERIAL AND METHODS

Rabbits

Female albino rabbits weighing between 5 and 4 5 kg were used

The left kidney of each rabbit was removed for two reasons (*a*) We could thus be sure that the study was carried out on rabbits with normal kidneys as judged by light microscopy and (*b*) We made the serum creatinine content a more sensitive parameter for changes in the glomerular function of the remaining kidney

Thrombin Each rabbit received thrombin (Topostasin Roche) in a dosage of about 300 units kg body weight dissolved in 45 ml NaCl 0 9 by infusion over a period of two hours into a lateral ear vein

Epsilon amino caproic acid (EACA) Preliminary experiments strongly suggested that EACA strengthened the pathological effect of intravascular coagulation on the kidney EACA itself did not cause histological renal lesions (six rabbits) For two to seven days before the thrombin infusion two intraperitoneal injections of a 10 solution of EACA were given up to 5 g EACA per day Just before the infusion a final intraperitoneal dosage of 20 ml 10 EACA solution was administered During the time that intraperitoneal injections were given the drinking water also contained EACA at a strength of 1 g per 100 ml

Blood pressure Systolic blood pressure was measured by the method of Grant and Rothchild (4)

Immunofluorescence The IgG fraction of a sheep anti rabbit fibrinogen serum was coupled to fluorescein iso thiocyanate (FITC) The specificity of immunofluorescent staining was shown by the absence of staining when the conjugate was previously absorbed with rabbit fibrinogen

Histology Renal tissue specimens were fixed in Zenker s fluid or in 10 buffered formalin (pH 7 35) and routinely embedded in paraffin Four μ sections were then stained hematoxylin eosin (HE) periodic acid Schiff (PAS) Masson Goldner trichrome (MGT) and phosphotungstic acid hematoxylin (PTAH) Sections cut at 1 μ were stained with Jones periodic acid silver methenamine (PASM) The sections were examined independently by at least two of the authors

Factor V (proaccelerin) was determined according to Stormorken s (14) modification of Owren s method (11)

Fibrinogen was estimated by the method of Strengers and Asberg (15)

Creatinine was measured according to de Vries and van Daatselaar (18)

RESULTS

All rabbits receiving thrombin showed diffus intravascular blood coagulation as measured by

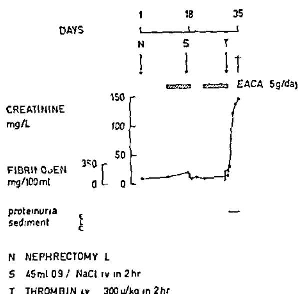


Fig 1 Creatinine levels in a rabbit dying in anuria after an episode of diffuse intravascular coagulation with renal cortical necrosis *The decrease in fibrino en level* during the thrombin infusion is indi ated in the figure by the perpendicular line below the letter T

a decrease of fibrinogen and factor V levels The consequences of this coagulation varied consider ably *however from animal to animal Many* animals died within eight days (group I) other rabbits survived but developed transitory impair ment of kidney function (group II) Several not only survived, but also showed no impairment of kidney function either during or after the throm bin infusion (group III) No change in systolic blood pressure was recorded

Group I (27 rabbits)

From the beginning of the thrombin infusion *there was a rapid rise of serum creatinine values* Most animals became anuric and expired within one to eight days (Fig 1) Renal cortical and in most instances medullary necrosis was found at autopsy and histological examination revealed ex tensive deposition almost exclusively in the ca pillary lumina of the glomeruli and rarely in small vessels of a material with the staining prop erties of fibrin When cryostate sections were flooded with FITC coupled sheep anti rabbit fibrin serum this material became fluorescent

Group II (11 rabbits)

During the first few days after the onset of intravascular coagulation these rabbits resembled those of the first group as far as kidney func tion was concerned A rapid increase of the serum creatinine content was observed but values fell to normal as quickly as they had risen except in *one case (Fig 2)*

In contrast with those of group I only one animal became anuric the anuria lasting for

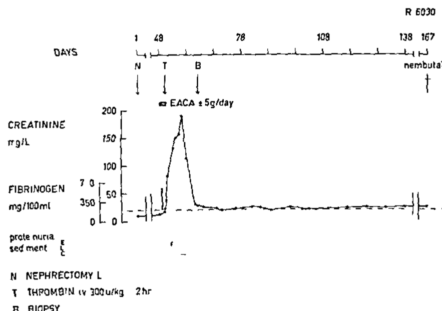


Fig 2 Case 1 in Table I Blood creatinine levels in a rabbit surviving a short episode of diffuse intra vascular coagulation The decrease in fibrinogen level during the thrombin infu sion is indicated in the fig ure by the perpendicular line below the letter T At biopsy (*B*) a histological diagnosis of renal cortical necrosis was made At autopsy chronic pyelone phritis like lesions were found The creatinine level never regained the pre infu sion value

Table I *Relationship between maximal rise in creatinine content and observed renal pathology at autopsy*

The severity of macroscopical and microscopical lesions is subjectively graded as − ± + ++ and +++
Glomerular pathology concerns the glomeruli in areas without interstitial fibrosis and tubular widening

Case	Maximal rise in creatinine content	Macroscopy		Microscopy			Months after infusion
		Enlargement	Surface	Glom	Tubuli	Fibrosis	
1	173	+++	+++	+	+++	++−	3 9
2	151	+++	+	±	+++	++	1 3
3	120	±	+++	++	+++	+++	0 7
4	105	+++	++	±	++	++	2 7
5	90	−	±	+	+	+	0 7
6	73	−	±	±	−	±	2 5
7	53	+	+	±	++	+++	1 2
8	33	±	±	−	+	+	2 8
9	24	±	±	±	+	++	1 4
10	16	−	−	±	−	−	1 2
11	12	++	±	±	++	+++	3 9
12	6	−	−	±	±	−	1 6
13	4	−	−	−	−	−	0 9
14	−	−	−	±	±	−	1 4
15	−	−	−	−	−	−	0 8
16	−	−	±	−	−	−	2 0
17	−	−	−	−	−	−	1 6
18	−	−	−	−	−	−	1 5

three days Abnormalities of the urine (presence of erythrocytes leucocytes casts protein) were uncommon even during impairment of renal function. The rabbits were killed by means of Nembutal 0 7 to 3 9 months after the thrombin infusion At autopsy the kidney was found to be enlarged in some cases and the surface showed flat retractions (Table I and Fig 3)

Of the histological changes (Table I) interstitial fibrosis was the most impressive In some cases it



Fig 3 Case 4 in Table I *Left* normal kidney of rabb t 1 month after unilateral nephrectomy *Right* kidney of rabb t 1.3 months after an ep ode of ntra ascular coag ulat on Irregular surface nd enla gement

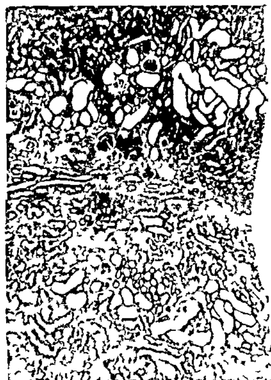


Fig 4 Case 4 n Table I Kidney of rabb t 1 3 months after a short ep sode of intravascular coagulation. Radial fibros s and cyst like dilatation of adjacent tubuli. MGT stain org magn × 35

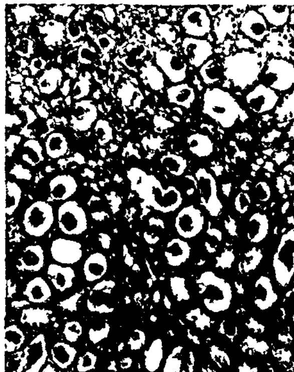


Fig 5 Case 3 in Table I Kidney of rabbit 0 7 months after an episode of intravascular coagulation Thyroid like appearance PAS stain org magn × 106



Fig 6 Case 4 in Table I Kidney of rabbit 1 3 months after a short episode of diffuse intravascular coagulation Moderate mononuclear infiltration and varying degrees of glomerular damage PAS stain org magn × 113

was diffuse throughout the kidney Radial locali zation from cortex to medulla alternating with relatively normal areas was frequently observed (Fig 4) In some instances small areas of kidney had totally been replaced by connective tissue Another conspicuous feature was the widening of the tubular lumina in the cortex and medulla with flattening of the epithelium of tubules They were found particularly along the interstitial fibrosis Within the areas of interstitial fibrosis on the other hand the tubules were smaller with a thickening of basement membranes Casts were present in varying numbers mainly in the m dulla (Fig 5)

Glomerular pathology was most pronounced in and along the interstitial fibrosis Many glomeruli had been totally destroyed and the less seriously affected showed an increase of fibrillar material in the mesangium proliferation of intracapillary cells adhesions and periglomerular fibrosis In some glomeruli Bowman s space was enlarged while the capillary network appeared to have shrunk In areas with no interstitial fibrosis the mo t that was found was some cell proliferation and an increase of PAS positive material in the mesangium Mononuclear infiltration of the in terstitium was not characteristic of th se kidn ys although it was seen in two (Fig 6) In thre rabbits (Table I cases 1 2 and 4) open kidney biopsies were performed 10 to 14 days after the thrombin infusion At that time the enlargement of the kidney subsequently found at autopsy was not noticed In one case (Table I case 1) a diagnosis of cortical necrosis was made Neither in the autopsy renal specimens nor in the biopsies was fibrin detectable

Group III (7 rabbits)

The animals in this group were treated exactly the same as in groups I and II they are char acterized by surviving the intravascular coagula tion without functional damage to the kidney the criterion for functional damage being a rise of the creatinine level of more than 10 mg/l serum 24 hours after the thrombin infusion In

these rabbits renal pathology was absent or minimal Table I correlates the severity of kidney function disturbance expressed as the maximal rise in the serum creatinine content after intravascular coagulation and the severity of the kidney lesions found at autopsy

DISCUSSION

Renal cortical necrosis as a result of experimental diffuse intravascular coagulation has been thoroughly studied in the past The renal pathology of animals surviving intravascular coagulation has not however hitherto been sufficiently described The only relevant publication is that of Vassalli et al (16) They analysed the glomerular changes following fibrin deposition in the rabbit kidney by electron microscopy the maximal time between fibrin deposition and investigation being 14 days It seemed probable that fibrin deposits not only caused acute damage to the glomeruli but could also totally destroy glomeruli Our own results can be summarized as follows After a period of diffuse intravascular coagulation induced by thrombin infusion one group of animals died within eight days and renal cortical necrosis was found This outcome could be predicted not only by the increase in the serum creatinine content but also by the presence of free haemoglobin in the plasma immediately after infusion and a visible hyperlipemia 24 hours after the infusion (1) Other rabbits (groups II and III) did not die spontaneously and were killed at various intervals after the thrombin infusion It is important to note that at that time there was no reason to suppose renal pathology for serum creatinine values were normal urinalysis revealed no abnormalities and the systolic blood pressure was not raised Nevertheless in some animals macroscopical and microscopical renal pathology was present which correlated reasonably well with the changes in kidney function immediately after the period of intravascular coagulation The distribution of the histological damage was patchy It consisted mainly of interstitial fibrosis widening of the tubules and variable glomerular damage in and along the interstitial fibrosis In some cases there was cast formation and interstitial mononuclear infiltration This seems to be the result of a series of processes initiated by intravascular coagulation Circulating fibrinpolymers are phagocytized by the reticulo-endothelial system (7) but when this system is saturated they may lodge in small vessels especially in the glomerular capillaries and give rise to impairment of renal blood supply Renal ischaemia then develops This may be relatively harmless when fibrin is quickly removed by fibrinolysis triggered off for example by urokinase probably produced by the glomerular capillary wall (17) Necrosis ensues from ischaemia of longer duration The necrotic tissue will be replaced by connective tissue The transient rise in serum creatinine and the observed ultimate renal pathology of rabbits that survive intravascular coagulation can be understood if one assumes that ischaemia of variable duration has been present in various parts of the kidney The situation at the level of the interlobular arteries may have been decisive for the characteristic radial distribution of the interstitial fibrosis Tubules will be obstructed by retraction of the connective tissue leading to internal hydronephrosis" (10) This may explain the cyst like tubules with flattened epithelium and casts the widened Bowman s space of some glomeruli and the enlargement of the total kidney volume

As outlined before diffuse intravascular coagulation in humans may also occur in many conditions and it seems reasonable to expect that renal pathology comparable with that obtained in our experiments may develop in some patients In all probability this renal pathology marked by patchy distribution of the lesions interstitial fibrosis widened tubules with casts (thyroid like structure) periglomerular fibrosis and sometimes mononuclear infiltration would be classified histologically as inactive chronic pyelonephritis It must be realized that in our rabbits bacterial infection did not contribute to the kidney damage Several authors have stressed that in man there is often a failure to establish any causal relationship between the morphologic changes of chronic pyelonephritis" and bacterial invasion of the kidney (5 6) Bacteriological cultures of kidney tissue (2 12) or urine (12) appear to be positive only in a small number of patients with a histologically confirmed diagnosis of chronic pyelonephritis It is therefore highly probably that renal pathology called "chronic pyelonephritis" has very different aetiologies (3) It may sometimes follow an episode of intravascular coagulation

weakness He could not walk unaided For several months he had required constant analgetics and was bound to a wheelchair by severe pains and muscular fatigue in the legs and hip joints

The diagnosis of hypophosphatemic osteomalacia type II (4) was made On the basis of the characteristic features of the case osteomalacia with proximal myopathia renal glycosuria in the absence of renal aminoaciduria and a blood chemistry typical of hypophosphatemia (serum phosphorus 1 1 mg % serum calcium 5 0 mEq and alkaline phosphatase moderately increased to 6 6 Bessy Lowry units)

The patient was treated with large doses of vitamin D_2 inorganic phosphate and calcium under the control of full metabolic balance investigations of calcium and phosphorus metabolism for several months and was considered to be fully rehabilitated after about one year of therapy as described in detail elsewhere (7)

The detailed trace element balance studies on subject B reported in the present paper were performed during two stages in the course of the disease

I Immediately after admittance when the patient was severely crippled after two years on the insufficient vitamin D_2 substitution dose of 4800 IU/day and showed a negative balance with respect to calcium and phosphorus metabolism

II After about three months of treatment when a marked improvement was apparent (no pains increased muscular strength ability to walk visible callus in the Milkman fractures) a markedly positive calcium and phosphorus balance had been achieved and the substitution treatment consisted of vitamin D 545 000 IU/day (Spir Fortedol) calcium 3 g (Calcium, Sandoz, effervescent tablets) and 15 g of sodium monophosphate

Subject C

A 58 year-old man with a long history of anemia and other signs of malabsorption This is indicated by the following diagnoses made at various hospitals—1935 pernicious anemia 1945 sideropenic anemia 1953 hematuria 1963 hemolytic anemia, 1964 anemia (folic acid deficiency + hemmorhagia diathesis) Later in 1964–1965 the patient developed signs of proximal myopathy hypocalcuria, hypomagnesemia hypocalcemia tetany and marked osteopenia (no Milkman fractures visible) A very severe steatorrhea amounting to about 70 of the fat intake as well as other signs of intestinal malabsorption such as marked atrophy of the jejunal mucosa (Crosby capsule biopsy) and the snow flake" signs (X ray) have been found more recently The patient is now considered to be a severe case of idiopathic non tropical sprue with malabsorption of a broad spectrum of food, vitamins and nutritive salts

The present substitution therapy of the patient has been adjusted in several steps during the last year and consisted at the time of our study of the following substitution vitamin D 150 000 IU (spir Fortedol) ferrous sulfate 1 5 g folic acid 15 mg (Folacin) vitamin B-complex (Akvavimin, three tablets) vitamin K 10 mg (K vimin) per day and vitamin B_{12} 500 μg/month (Cykobemin)

On this treatment it has so far been possible to keep the patient in a good general condition in spite of his very high need for calories (about 4000 cal./day under hospital conditions) and certain difficulties in maintaining his weight when performing his ordinary work (gardening) For this reason a number of attempts (so far not very successful) have been made under the control of full balance studies of fats, nitrogen, calcium and phosphorus to decrease his steatorrhea by regulating his diet (fat poor gluten free short-chain fatty acids)

The detailed trace element balance studies on subject C were performed under the following two conditions:

I With the patient on a normal hospital diet with the above mentioned substitution therapy and 1000 μg of vitamin B_{12} daily

II With the patient on the same substitution therapy minus B and with 90 g of the fat in the diet replaced by 80 g of short-chain fatty acids (15)

Balance technique

The subjects A B and C (see below) were kept on constant diet prepared at the metabolic ward and all feces and urine were collected for balance periods of five days The conventional carmine-labeling technique was applied (5) The loss of trace elements in urine and feces for each balance period was determined using techniques described below on aliquots of urine and homogenates of feces The intake of trace elements was determined by analysis of homogenates of duplicates of the daily menu All drugs given to the patients (B and C) were added to the model diet before homogenization. The balance data obtained were charted according to Reifenstein et al. (6)

Neutron activation analysis

The different specimens collected were kept in polythene bags cooled to −20 C When required for analysis, aliquots were transferred to quartz ampoules, which were weighed dried and sealed as previously described (11) The ampoules were then irradiated together with standards in the R 2 reactor at Studsvik with a thermal neutron flux of 2 10^{13} n/cm /sec for 24 hours. A decay interval of two days elapsed before chemical processing. The irradiated ampoules were chilled in fluid nitrogen before opening to reduce the pressure induced during irradiation The chemical separation of the elements includes destruction of the organic material with H_2SO_4 and H_2O_2, a distillation step with HBr and separation on different ion-exchange columns into 14 groups of elements (10 14)

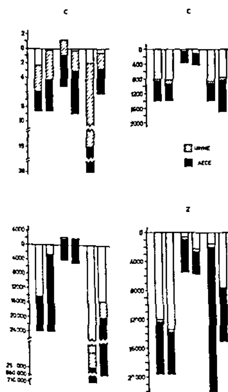
The γ-spectrometric measurements were carried out with a transistorized 512-channel pulse height analyzer attached to a 3" × 3 NaI (Tl) well type crystal The identification and the quantitation of the elements have also been described earlier (10 11)

RESULTS

The trace elements studied have been divided into three groups the trace elements with known biological function (Table I and Fig 1) the trace

Table I *Trace elements with known biological function*
Amounts in µg/24 h

Element	Subject	Period	Intake	Excretion Feces	Excretion Urine	Balance
Co	A	I	8 6	2 7	3 6	+2 3
		II	8 6	4 3	4 1	+0 2
	B	I	5 2	4 3	2 1	−1 2
		II	8 9	5 8	2 8	+0 3
	C	I	38	19	17	+2
		II	6 1	3 4	2 1	+0 6
Cu	A	I	1 400	550	42	+808
		II	1 400	460	110	+830
	B	I	370	310	55	+5
		II	420	280	26	+114
	C	I	1 400	470	58	+872
		II	1 700	870	73	+757
Fe	A	I	24 000	9 400	290	+14 310
		II	24 000	21 000	320	+2,680
	B	I	4 100	5 700	590	−2 190
		II	5 000	6 600	180	−1 780
	C	I	710 000	460 000	4250	+245 750
		II	660 000	640 000	4500	+15 500
Zn	A	I	19 600	7,200	400	+12,000
		II	19 600	5 800	390	+13 410
	B	I	5 500	4 500	410	+590
		II	5 700	3 100	380	+2 200
	C	I	22 000	20 000	470	−1 530
		II	15 000	7 300	150	−7 550



elements with suspected biological function (Table II and Fig 2) and the trace elements without known biological function (Table III) In Tables I–III columns 1–3 show the elements the subjects and the periods studied Column 4 shows the daily intake by ingestion columns 5 and 6 the daily excretion (feces and urine respectively) and column 7 the intake minus the excretion

The healthy control subject A showed a positive balance of the four trace elements with known biological function Co Cu Fe and Zn Subject B the patient with hypophosphatemia plus osteomalacia had a negative Co balance before institution of substitution therapy and a positive Co balance after The Fe balance of subject B was negative before as well as after the therapy whereas the Cu and Zn balances were somewhat more positive on treatment Subject C had a positive Co Cu Fe and Zn balance in the two situations studied

The three subjects studied showed different

Fig 1 Trace elements with known biological function Amounts in µg 24 hours.

Table II *Trace elements with suspected biological function*
Amounts in μg/24 h

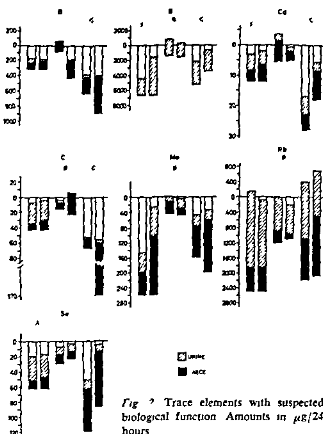
Element	Subject	Period	Intake	Excretion Feces	Excretion Urine	Balance
Ba	A	I	320	84	66	+170
		II	320	100	37	+183
	B	I	80	120	16	−52
		II	430	210	36	+184
	C	I	640	210	42	+388
		II	910	470	32	+408
Br	A	I	6700	33	2300	+4367
		II	6700	26	5200	+1474
	B	I	1400	35	2200	−835
		II	1600	45	1800	−245
	C	I	5300	27	3100	+2178
		II	3500	73	2800	+627
Cd	A	I	12	3 6	5 3	+3 1
		II	12	5 6	4 5	+1 9
	B	I	5 4	6 8	2 3	−3 7
		II	5 2	3 1	1 3	+0 8
	C	I	8	4 9	6 0	+17 1
		II	18	9 3	2 9	+5 8
Cr	A	I	43	8 1	27	+7 7
		II	43	13	26	+4
	B	I	16	9 2	3 0	+3 8
		II	23	27	1 8	−5 8
	C	I	67	13	2 1	+51 9
		II	170	110	4 2	+55 8
Mo	A	I	260	63	51	+146
		II	260	160	75	+25
	B	I	44	31	28	−15
		II	48	18	25	+5
	C	I	160	85	29	+46
		II	200	140	27	+33
Rb	A	I	2500	630	2000	−130
		II	2500	620	1800	+80
	B	I	1200	300	900	±0
		II	1100	110	780	+210
	C	I	2200	1100	1500	−400
		II	2100	1600	1200	−700
Se	A	I	62	11	31	+20
		II	62	15	30	+17
	B	I	29	12	10	+7
		II	23	10	10	+3
	C	I	120	57	12	+51
		II	87	74	8 5	+4 5

balance patterns for the trace elements with suspected biological function Subjects A had a positive or normal balance of all elements Subject B showed a negative balance of Ba Cd and Mo before treatment and a positive balance after with positive Rb and Se balances and a negative Br balance before as well as after treatment The balances of subject C were all positive except for the Rb balance which was markedly negative in both periods The balances given in Table III of some of the trace elements without known biological function (As Au Ce Cs Hg La Sb Sc Sm W) are due to technical failure not complete in all subjects and periods They show no consistent differences between the patterns in the various subjects and balance periods

Table III *Trace elements without known biological function*

Amounts in µg/24 h

Element	Subject	Period	Intake	Excretion Feces	Excretion Urine	Balance
As	A	I	8 4	1 7	7 3	−0 6
		II	8 4	4 1	5 7	−1 4
	B	I	66	2 5	68	−4 5
		II	170	1 9	46	+122 1
	C	I	15	7 1	23	−5 1
		II	130	21	32	+77
Au	A	I	0 13	0 020	0 014	+0 096
		II	0 13	0 064	0 050	+0 016
	B	I	0 092	0 066	0 040	−0 014
		II	0 22	0 031	0 070	+0 119
	C	I	0 037	0 058	0 0014	−0 0224
		II	0 10	0 067	0 0024	+0 0306
Ce	A	I	62	19		
		II	62	21	40	+1
	B	I	56	8 3	31	+16 7
		II	47	5 9	21	+20 1
	C	I	48	12	13	+23
		II		56	5 2	
Cs	A	I	13	2 1	8 8	+2.1
		II	13	1 7	11	+0 3
	B	I	5 2	0 65	4 2	+0 35
		II	4 2	0 40	3 2	+0 60
	C	I	15	3 8	6 0	+5 2
		II	30	12	10	+8 0
Hg	A	I	27	16	2 4	+8 6
		II	27	20	3 4	+3 6
	B	I	4 8	3 6	2 1	−0 9
		II	5 8	3 6	1 6	+0 6
	C	I	16	5 7	1 2	+9 1
		II		6 5		
La	A	I	8 6	1 8	0 25	+6 55
		II	8 6	2.2	0 30	+6 10
	B	I	0 99	1 9	0 15	−1 06
		II	0 85	0 29	0 10	+0 46
	C	I	17	2 7	0 50	+13 8
		II	15	4 3	0 73	+9 97
Sb	A	I	12	4	4	+4
		II	1?	11	3	−2
	B	I	9 6	10	1 8	−2 2
		II	15	11	2.2	+1 8
	C	I	16	9 9	2 2	+3 9
		II		12	1 9	
Sc	A	I	1 1	0 23	0 03	+0 84
		II	1 1	0 11	1 1	−0 11
	B	I	0 021		0 036	
		II	0 062		0 008	
	C	I	0 31	1 8	0 17	−1 66
		II	0 48		0 08	
Sm	A	I	22	7 8	3 1	+11 1
		II	22	8 7	2 4	−11 0
	B	I	1 7	6 7	0 15	−5 15
		II	1 6	4 4	0 12	−2 92
	C	I	130	40	1 4	+88 6
		II	23	110	1 8	−88 8
W	A	I	65	4 4	5 3	−55 7
		II	65	2 3	16	+46 7
	B	I	2 3	2 1	1 3	−1 1
		II	‑5	1 3	3 4	−2 2


Fig ? Trace elements with suspected biological function Amounts in $\mu g/24$ hours

DISCUSSION

Full balance studies with regard to trace elements in healthy subjects (8 12 13) or patients with metabolic diseases (13) are so far very scarce in the literature For this reason the following discussion must be limited to comparisons between the individuals and balance periods studied in the present paper

When comparisons are made between the normal subject (A) and the two patients (B and C) it is noted that with respect to trace elements with known and suspected biological function the former showed equilibrium or positive balances whereas the latter showed certain deviations from these patterns

In subject B the balances of several elements (Ba Cd Co and Mo) were negative before treatment (period I) and positive during treatment (period II) suggesting that treatment with vitamin D calcium, and phosphate might influence not only the calcium and phosphorus balances but also certain trace element balances Some indications were also obtained that to some extent treatment influenced the balances of Br Cu and Zn On the other hand the Fe balance of subject B was negative and uninfluenced both before and during treatment indicating that the substitution therapy was inadequate for iron This was confirmed by the clinical course of the patient (2) who developed a sideropenic anemia while under observation in the hospital The high phosphate dose given was considered to be partially responsible for the malabsorption of iron Other studies (2) have indicated that this may occur

In subject C most trace element balances were positive in both periods studied suggesting that the substitution therapy for these elements was satisfactory A notable exception to this rule was Rb which for unknown reasons in this case showed a negative balance The very high intake and excretion of Fe in both periods and of Co in period I reflects the substitution therapy given

The large variation in the balances of the trace elements without known biological functions seems to agree with the large variability in concentration of these elements in human tissues (7 10 11)

The present pilot study on trace elements might be influenced not only by a particular metabolic disorder but also by the conventional treatment in such cases

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GASTRIC HISTOLOGY AND AUTOANTIBODIES IN PERNICIOUS ANEMIA

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Abstract The gastric histology and occurrence of anti nuclear factors and circulating autoantibodies against intrinsic factor parietal cell and thyroid cell cytoplasm and mitochondria were studied in 78 patients with Addisonian pernicious anemia in remission The data were analysed with respect to relations between gastric histology autoantibodies in serum age and sex of patients and duration of pernicious anemia

Intrinsic factor antibody was not found in patients with gastric atrophy who had a slightly higher mean age than was found in the patients with atrophic gastritis

Parietal cell antibody was found in all patients below 60 years of age but apart from these findings no clear cut relations could be derived from the data

The significance of the gastric autoantibodies is discussed It is concluded that intrinsic factor antibody secreted into gastric juice may neutralise intrinsic factor and that the production of this antibody may be the crucial factor in the evolution of overt pernicious anemia So far no direct evidence has been produced to show that the gastric autoantibodies play a role in the pathogenesis of the gastric lesion

The introduction of a flexible suction gastric biopsy tube (43) made available a simple and relatively safe method of obtaining fragments of the gastric mucosa for histological study and the stomach in pernicious anemia (PA) has been intensively investigated by this method It is generally agreed that the gastric mucosa in this condition invariably shows severe pathological changes in the form of pronounced atrophy of specific glands and some degree of cellular mucosal infiltration with lymphocytes and plasma cells but it has been much discussed whether "gastric atrophy or "atrophic gastritis is the typical lesion in PA (23 24 26 33 40 42) The differing opinions are probably mainly accounted for by differing criteria for these two conditions (23 40) The discussion is of particular interest in connection with Faber s hypothesis that gastritis is a progressive disease eventually developing into a final state accompanied by PA (11) a hypothesis still not proved nor disproved

The etiology of PA is still unknown but the well recognised association of this disease with gastric cancer the familial incidence of both diseases and the predominance of blood group A in both strongly suggest a predisposing genetic factor for the development of the gastric lesion (5 27)

It is by now accepted that there is a genuine association between PA and thyroid diseases probably of autoimmune origin (10 41) The histological association between atrophic gastritis in PA and lymphoid thyroiditis is of interest in the light of the possibly autoimmune mechanisms in PA suggested by Taylor and by Schwartz (30 34) Independently these authors demonstrated in sera from untreated PA patients a substance able to block the effect of intrinsic factor in vivo and thought that this substance might b an autoantibody to intrinsic factor Abels et al (1) showed that it was a 7 S gammaglobulin and these authors as well as others proved that this intrinsic factor antibody (IFA) could be demonstrated by in vivo as well as by in vitro methods (2 20 31 36) IFA is reported with a frequency of 33–59 in large series of PA patients studied by in vitro methods without any clear-cut clinical differences between IFA positive and negative patients (1 8 15 17 31 36) This antibody is found almost exclusively in PA patients (having the disease in manifest or latent form) (13 17 37 38)

Another gastric autoantibody (also a 7 S gammablobulin but different from IFA) directed against microsomal fractions of the parietal cell

was described in 1962 (18 36) demonstrable by a complement fixation reaction or the more sensitive immunofluorescence technique This parietal cell antibody (PCA) is found in 73–89% of PA patients with a higher prevalence 93–96%, in patients below 60 years of age than in patients above this age 76–82% (8 15 16 21 28 36) PCA is also found in gastritis patients without PA but in a considerably lower frequency and in 2–16% of healthy subjects although it is doubtful whether PCA is found in subjects with a normal gastric mucosa (8 13 28 37 44) Patients with PCA in serum especially those with PA, have frequently also thyroid autoantibodies in serum and patients with thyroid diseases show a high prevalence of PCA and if they concomitantly have latent or manifest PA IFA is a relatively common finding (8 38 41)

Patients with Addisonian PA are achlorhydric subjects with inadequate secretion of intrinsic factor and severe pathological changes in the gastric mucosa Comparatively little however is known about the state of the gastric mucosa (the target organ for the autoantibodies if they are of importance in the *pathogenesis* of the gastric lesion) in relation to the occurrence of circulating gastric autoantibodies in these patients (22 29 37 44) We report here our findings on gastric histology and autoantibodies in relation to age and duration of the disease in a series of PA patients and will discuss some aspects of the significance of the gastric autoantibodies

MATERIAL AND METHODS

Twenty-eight patients with Addisonian PA were studied (18 women ten men) with a mean age of 64 years (range 31–81) and a mean duration of the disease of four years (mean 0–18) The diagnosis had been established by appropriate means (including serum vitamin B_{12} assay Schilling test without and with oral hog intrinsic factor administration, bone marrow examination and in many patients augmented histamine test which in all cases revealed achlorhydria) At the time of examination all patients were in remission they had all responded favorably to treatment, usually with a depot vitamin B_{12} preparation

Each patient had had a gastric biopsy and at the same time a blood sample was drawn for determination of IFA, PCA thyroid cytoplasm antibody (TCA) the mitochondrial antibody (MCA) and the antinuclear antibody (ANF)

Gastric biopsy

The biopsy was done with a Crosby capsule (9) from the body or fundus of the stomach after the capsule had been sited under X ray screening Sections were stained with hematoxylin-eosin and PAS The histological appearances were graded blind into the following three groups

Moderate atrophic gastritis (MAG) Pronounced atrophic gastritis with diffuse round cell infiltration Some gastric glands were seen consisting of cuboidal cells with basally compressed nuclei occasionally chief and parietal cells Intestinal metaplasia absent or slight

Severe atrophic gastritis (SAG) Mucosal and gland atrophy more pronounced than in MAG Round cell infiltration always present Glandular compartments seldom seen (of the type as in MAG) no chief or parietal cells Often widespread intestinal metaplasia

Gastric atrophy (GA) Severely atrophic mucosa *without* round cell infiltration Only few and extremely atrophic glands without any specific cells Widespread intestinal metaplasia

Measurement of IFA

IFA was determined radioimmunologically (31) by a modification of the serum-charcoal method (2) A titer ≥ 5 mμg vitamin B_{12} was considered positive for IFA

Measurement of PCA TCA MCA and ANF

PCA TCA and MCA were determined by the fluorescent antibody technique using human gastric mucosa thyrotoxic gland and human renal cortex respectively as antigens Anti nuclear factors (ANF) were determined simultaneously

The undiluted sera were incubated with the antigens for 30 minutes at 20 C after washing in buffered saline the tissue sections were incubated with FITC labeled anti human gammaglobulin and mounted in buffered glycerine The microscope was a Leitz Ortholux (Zernicke)

The interpretation of the readings was as follows Staining of the cytoplasm of the thyroid and parietal cells but no staining of the renal tubules was considered indicative of the presence of organ specific thyroid and gastric antibodies Though the fluorescence given by the mitochondrial antibodies of the cytoplasm of the parietal thyroid and renal tubule cells is often coarsely granular the presence of this antibody may make it difficult to establish the eventual coexistence of the organ specific antibodies by the immunofluorescent technique absorption of such sera with rat liver mitochondrial and mi rosomal tissue fractions has been necessary in five cases in the present report

The positive frequency for PCA TCA MCA and ANF in a control material of 84 individuals matched for sex and age was 5 18% 2 and 8 respectively

Presentation of results

In Tables I–VII the abbreviations MAG SAG and GA are used for the histological findings IFA PCA TCA MCA and ANF are used for the antibodies (vide supra) age and duration of the disease are given in years (mean values followed by ranges in brackets) and F/M stands for female/male ratio

Table I *Findings at gastric histology in relation to positive antibody reactions*

Gastric histology	No of pat	Age	Duration of PA	F/M	No of positive antibody reactions IFA	PCA	TCA	MCA	ANF
MAG	14	61 (31-81)	4 (0-18)	9/5	6	12	6	1	0
SAG	9	64 (47-75)	4 (0-11)	7/2	7	8	3	3	1
GA	5	71 (54-80)	4 (0-8)	2/3	0	4	0	1	0
Total	28	64 (31-81)	4 (0-18)	18/10	13	24	9	5	1
					46	86	3	18	4

Table II *Age of patients at time of investigation in relation to gastric histology and positive antibody reactions*

Age between	Mean age	No of pat.	F/M	Gastric histology MAG	SAG	GA	No of positive antibody reactions IFA	PCA	TCA	MCA	ANF
31-66	54	14	11/3	8	5	1	6	13	5	3	0
67-81	75	14	7/7	6	4	4	7	11	4	2	1

RESULTS

Our results are summarized in Tables I–VII

With the three histological sub groups used the average age of the patients increased slightly with the severity of the gastric lesion and IFA was not found in any of the five patients with GA while the prevalence of PCA was the same in the three groups (Table I) The patients were divided into two groups according to age (Table II) and the prevalences of IFA and PCA in these groups were similar All ten patients below 60 years of age had PCA in serum in the younger group only one patient showed GA while this was seen in four patients in the older group Divided into groups with a duration of PA *less* and *more* than four years respectively the patients showed no difference in gastric histology or prevalence of IFA and PCA (Table III) According to age at onset of PA (i e the time when the diagnosis was established) the 28 patients were divided into two groups in the 14 patients who were below 60 years when PA was diagnosed only one had GA but the prevalence of IFA and PCA did not differ in the two groups (Table IV) As a group the 18 females (Table V) had a lower mean age and three of the ten males (aged

Table III *Duration of overt PA in relation to gastric histology and positive antibody reactions*

Duration	No of pat	Gastric histology MAG	SAG	GA	No of positive antibody reactions IFA	PCA	TCA	MCA	ANF
Less than 4 y	13	6	4	3	5	11	3	2	0
Four y or more	15	8	5	2	8	13	6	3	1

Table IV *Age of patients at onset of PA in relation to gastric histology and positive antibody reactions*

Age at onset	Mean age	No of pat	F/M	Gastric histology MAG	SAG	GA	No of positive antibody reactions IFA	PCA	TCA	MCA	ANF
Below 60 y (30-59)	49	14	10 4	8	5	1	7	12	4	3	0
Above 60 y (6 80)	70	14	8 6	6	4	4	6	12	5	2	1

Table V *Gastric histology and positive antibody reactions in females and males*

No of pat	Age	Duration of PA	Age at onset	Gastric histology			No of positive antibody reactions				
				MAG	SAG	GA	IFA	PCA	TCA	MCA	ANF
Females											
18	59 (31–81)	3 (0–11)	56 (30–80)	9	7	2	10	16	7	5	1
Males											
10	71 (57–80)	5 (0–18)	66 (51–80)	5	2	3	3	8	2	0	0

Table VI *Positive and negative antibody reactions in relation to age and duration of PA and gastric histology*

	No of pat	Age	Duration	Gastric histology		
				MAG	SAG	GA
IFA positive	13	64 (45–80)	4 (0–18)	6	7	0
IFA negative	15	64 (31–81)	4 (0–11)	8	2	5
PCA positive	24	63 (31–81)	4 (0–11)	12	8	4
PCA negative	4	69 (60–76)	7 (0–18)	2	1	1

Table VII *Combinations of the two gastric autoantibodies in relation to age duration of PA and gastric histology*

IFA	PCA	No of pat	Age	Duration	MAG	SAG	GA
No	No	2	(60–67)	(1–9)	1	0	1
Yes	No	2	(74–76)	(0–18)	1	1	0
No	Yes	13	64 (31–81)	4 (0–11)	7	2	4
Yes	Yes	11	62 (45–80)	4 (0–7)	5	6	0

76–80 years) had GA accordingly (Table I) relatively few positive reactions for IFA were found in the males

From Tables VI and VII it appears that the mean age in patients with and without IFA was identical that patients without PCA were at least 60 years of age and that PCA alone or PCA *and* IFA are the common findings in patients with PA two patients only had neither IFA nor PCA in serum

DISCUSSION

Williams et al (39) found that a single gastric biopsy was representative of the gastric mucosal state in 85–90° of cases and that gastric atrophy when found was always diffuse The criteria for histological classification used in the present study seem to correspond rather closely to those employed by Williams et al (40) who of 44 patients with PA (after single gastric biopsies) classified six as gastric atrophy and 38 as chronic atrophic gastritis The findings by these and other authors (4) that the degree of atrophy of the specific fundic glands is unrelated to the duration of the disease are confirmed by the results in the present study

The percentages of positive reactions of IFA and PCA in the present series of PA patients correspond to those reported in the literature ANF is uncommon in PA but the percentage of positive reactions for thyroid antibodies is somewhat higher in the literature than reported here though likewise most frequent in females (1 8 15 17 31 35 36) In the present report no thorough investigation of liver function was performed which might explain some of the MCA positive reactions hitherto mainly found in cirrhosis Our findings of prevalence of IFA and PCA in relation to age and PA duration correspond to findings in larger series (31 36)

In patients with atrophic gastritis without PA

Table VIII *Prevalence of PCA and IFA (graded from + to + + +) in relation to gastric histology from recent reports on PA patients compared with present study*

			No classified as		Prevalence of			
					PCA		IFA	
Authors	Year	No of pat	*Atrophic gastritis (G)	Gastric atrophy (A)	G	A	G	A
Wright et al	1966	25	16	9	+ +	+ + +	−	+ +
te Velde et al.	1966	29	18	11	+ − +	+	−	− −
Jeffries et al.	1966	6	3	3	+ + +	+		
Rødbro et al.	1967	16	13	3	+ +	(−)	+ −	(+)
Present study		28	23	5	+ + +	− + +	+ −	(−)

a rough correlation between degree of mucosal atrophy, mucosal cellular infiltration and the presence of PCA in serum has been reported (8, 19) and Fisher et al (13) found a correlation between the degree of depression of gastric function (judged by vitamin B_1 absorption) and the prevalence of gastric autoantibodies

Relatively few studies have been published on the relation between gastric histology and the presence of IFA and PCA in serum in PA patients (22, 29, 37, 44). The findings in these reports are summarised together with our present results in Table VIII. The figures look rather bewildering. It appears that the frequency of IFA and PCA in PA patients with atrophic gastritis may be higher than *or* similar to *or* lower than in PA patients with gastric atrophy. The differences may partly stem from the differing criteria for histological classification (cf. Table I). For the results of PCA it should be noted that Jeffries et al (22) found a low titer of PCA in their patients with widespread intestinal metaplasia and a high titer in patients with little intestinal metaplasia, that Rødbro et al (29) only recorded the strongly positive PCA reactions as positive and that the other studies including the present report positive *or* negative reactions for PCA. Although it has been reported that the titer of PCA is unrelated to the duration of PA and that individual patients during a period of four years show no variation of titer it *may* be an advantage to determine PCA quantitatively (21).

It has been much discussed whether the gastric autoantibodies are secondary phenomena (i.e. markers of the gastric lesion) or whether they play an important role in the pathogenesis of the gastric lesion. Jeffries et al (22) found that PA patients with high titers of PCA retained a certain potential to regenerate parietal cells as shown by the reaction to corticosteroid treatment, such patients contrast with the PA patients with gastric atrophy and severe intestinal metaplasia, considered to represent a burnt out state of the gastric lesion, and the authors concluded that the autoantibodies probably were secondary phenomena. This opinion, also held with some modifications by te Velde et al (37), was partly confirmed by Rødbro et al (29) in their series of prednisone treated PA patients.

There are several objections to the view that gastric autoantibodies are *merely* secondary phenomena simply reflecting cell destruction in the gastric mucosa, although the evidence is still indirect. The gastric histological lesions in patients with severe atrophic gastritis who have adequate vitamin B_1 absorption are indistinguishable from the findings in PA (7, 23), achlorhydria may be found in both groups, but atrophic gastritis patients without PA apparently have a limited but presumably adequate intrinsic factor secretion, the frequency of PCA is lower than in PA, IFA is usually not found and development into overt PA is uncommon (8, 13, 15, 37, 38, 44). Patients with gastritis in the gastric remnant after subtotal gastrectomy and patients with gastric carcinoma who have severe atrophic gastritis only seldom have PCA in serum (13, 25). It should, however, be borne in mind that atrophic gastritis and gastric atrophy probably are common *morphological* manifestations of many different pathogenic mechanisms and it is *possible* that the gastric lesion in PA depends upon a genetically determined disorder of immunological tolerance of the organ specific type

Both PCA and IFA can be identified in gastric juice from PA patients (14 21) and IFA has also been found in saliva (6) Clearly the presence of IFA in gastric juice may play an important role in determining whether vitamin B_{12} absorption is sufficient when the intrinsic factor secretion is severely impaired In PA the gastric mucosa may still produce small amounts of intrinsic factor the biological activity of which may be inhibited by IFA either at the mucosal level or in the lumen of stomach or intestine The production of IFA may thus in several ways be the crucial factor in the evolution of overt PA and this production may be dependent upon a genetically determined liability to develop an autoimmune disorder No difference in vitamin B_{12} absorption is found between PA patients with and without IFA in serum (3) and the possibility that hog intrinsic factor in PA patients has a higher potentiating effect on B_{12} absorption than has human intrinsic factor (32) has been taken as evidence of an antibody to human intrinsic factor operating at cellular level in the small intestine (3) This is one attempt to explain why only 40–50% of PA patients have IFA in serum Other authors have speculated that the development of PA in the antibody negative individuals is due to some other mechanisms e g a cell mediated tissue destruction independent of humoral antibodies or progression of simple atrophic gastritis The test for IFA might be inadequate or the antibody might not appear in the blood due to combination with the antigen or IFA may have been present during the evolution of the gastric lesion and disappeared later (8 16 35 41)

The secretion of IFA into gastric juice may undoubtedly play a role in the physiology of vitamin B_{12} absorption since the antibody may neutralise intrinsic factor but it remains unsettled whether PCA and IFA have any part in the pathogenesis of the gastric lesion the evidence for this is so far only indirect and preliminary animal experiments have shown no depressive effect of PCA on gastric secretion (12) The findings by other authors for PCA (22 37) and the present findings for IFA indicate that antibody production may decline with advancing atrophy of the gastric mucosa which has been taken as support for the concept of these antibodies as secondary phenomena However large series of patients with atrophic gastritis with and without PA have to be followed for several years with repeated biopsies and quantitative measurement of autoantibody titer to elucidate this problem which is closely linked with the old riddles Is atrophic gastritis a progressive disease? and What is the etiology of pernicious anemia?

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REVERSIBLE RETINAL DETACHMENT IN RENAL INSUFFICIENCY

Report of Five Cases

Ib Steiness

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Abstract Revers ble retinal detachment was observed in five patients with renal failure and hypertension Two of the patients had undergone renal transplantation the others suffered from chronic glomerulonephritis It is concluded that hypertension was the primary cause of the detachment while fluid retention and uraemic intoxi cation were presumably contributory factors

The occurrence of serous retinal detachment in renal insufficiency is a rare and seldom detected complication and opinions on the pathogenesis are still divided Towards the end of last century several isolated cases were reported most often associated with advanced disease and generalized anasarca (3 5)

In his excellent account including 13 cases studied by himself Moore concludes that the liability to detachment is largely proportional to the severity of the retinal changes that the oc currence of detachment is not related to the presence or absence of general oedema but is due to a subretinal exudation probably entirely derived from the retina

Recently Lapco et al (2) have drawn attention to this complication in a report of eight cases All eight patients had chronic renal insufficiency hypertension and peripheral oedema No con sistent change was noted in the level of the blood pressure before or after the onset of the retinal detachment which however reversed with cor rection of water retention and dilutional hypo natraemia Local retroretinal oedema as part of a universal overhydration is therefore postulated as the cause of the detachment A similar case has been reported by Sharpstone and Lee (7) On the other hand Buchanan and Ellis (1) observed re solution of bilateral retinal separations in a 12 year-old boy on regular haemodialysis treatment after control of the hypertension by bilateral ne phrectomy

During the last two years we have seen retinal detachment in five patients with renal insuf ficiency and hypertension Below the case histo ries will be presented in chronological order The results of common investigations are shown in Tables I and II

CASE REPORTS

Case 1

J C (Fig 1) a ?1 year-old man underwent right nephrectomy after preoperative cobolt irradiation for a large malignant tumour originating from the kidney Seven months later an acute radiation nephritis developed in the remaining left kidney associated with malignant hypertension and peritoneal dialysis was twice required before renal allotransplantation and left nephrectomy could be performed on June 18 1965

Immed ately before transplantation the patient had oedema of the legs but he felt well and was ambulant Ophthalmoscopy revealed grade IV hypertensive changes with marked oedema of the retina and gross bilateral pap lloedema

The first 36 hours after operation d ures s amounted to more than 10 l and oedema disappeared The night after operation an unexpected and unexplained hypo natraemia was observed Serum sodium was 116 mEq/l and at control two hours later 115 but was rapidly cor rected with hypertonic sodium infusion

Hypertension pers sted after transplantat on and for several days the pat ent was comatose w th convuls ve ep sodes then gradually consciousness returned

Seven days after operation amaurosis was suspected and funduscop c examination showed almost complete bullous retinal detachment At this time the patient was undoubtedly somewhat ove hydrated but w thout peri pheral oedema Four days later there was an appreciable regress on of the detachment and after a week only small areas of detachment at the lower poles of each fundus rema ned These persisted unaltered at the last ophthalmoscopy ?4 days after transplantation

Table I *Summary of clinical data at diagnosis of retinal detachment*

Pat no	Sex	Age	Diagnosis	Duration of detachment (days)	B P (mm Hg)	Grade of hypertensive retinopathy (Keith Wagener and Barker)	Peripheral oedema	Clinical condition
1	♂	21	Renal transplantation	> 17 (died)	170/120	IV	0	Hypertensive encephalopathy
2	♂	46	Chronic glomerulonephritis	9	220/125	III IV	0	Uraemic intoxication
3	♀	14	Renal transplantation	> 41 (died)	170/110	IV	(+)	Hypertensive encephalopathy
4	♀	29	Chronic glomerulonephritis	30–50	175/110	IV	+	Uraemic intoxication
5	♂	22	Chronic glomerulonephritis	20	175/125	III	0	Hypertensive encephalopathy

Table II *Laboratory investigations at diagnosis of retinal detachment*

Pat no	Serum creatinine (mg/100 ml)	Blood urea (mg/100 ml)	Serum albumin/globulin (g/100 ml)	Serum sodium (mEq/l)
1	6 7	230	2 7/1 8	135
2	26 0	274	3 5/2 1	144
3	1 9	40	2 8/2 1	135
4	17 5	330	3 0/2 3	136
5	18 0	198	3 5/3 2	146

The postoperative course was stormy and the patient died 35 days after transplantation of gastro-intestinal haemorrhage and coli septicaemia

Case 2

A M (Fig 2) a 46 year old farmer was transferred to this hospital on July 1 1965 in terminal uraemia secondary to chronic glomerulonephritis There was no history of acute nephritis Proteinuria and microscopic haematuria had incidentally been found in 1958

For three years hypertension had been present and renal function was deteriorating The patient had only complained of headache and tiredness until the last few days when vision had been blurred

On admission he was somnolent with muscular twitching and a pericardial friction rub was heard There was no oedema but over the lower parts of both lungs fine rales were present

Visual acuity was reduced to finger counting immediately before the eyes The optic fundi showed grade

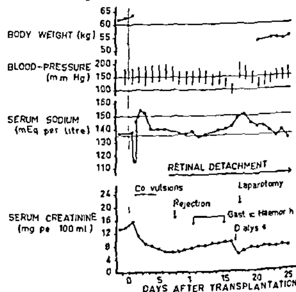


Fig 1 Case 1

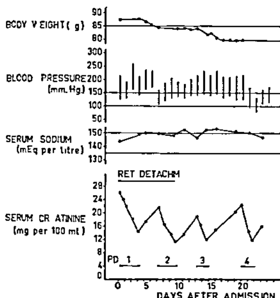


Fig 2 Case 2 PD = peritoneal dialysis

III IV hypertensive changes with blurring of the papillary margins and a marked oedema of the retina In the central parts of both fundi flat detachments were observed

Peritoneal dialysis treatment was instituted at once and two days later after termination of the first dialysis the detachments were strongly decreased in spite of unchanged body weight. Nine days later reattachment was total retinal oedema diminishing and finger counting normal

The general condition deteriorated gradually and the patient died 7 weeks after admission.

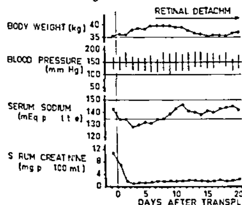


Fig 3 Case 3

Case 3

L M (Fig 3) a 14-year-old girl, was transferred to this hospital on April 30 1965 three months after an acute poststreptococcal glomerulonephritis with progressive deterioration of renal function and hypertension On admission the creatinine clearance was reduced to 7 to 8 ml/min and the B P was 180/130 mm Hg the optic fundi showed marked arteriolar narrowing and oedema but the discs were normal

From the beginning of June she was anuric and treatment with intermittent peritoneal dialysis was instituted In spite of antihypertensive therapy two acute attacks of hypertensive encephalopathy occurred and haemorrhages exudates and papilloedema developed in the fundi

Renal transplantation bilateral nephrectomy and splenectomy were carried out on August 24 However hypertension persisted and the night after the operation an episode of convulsions occurred.

Seven days after transplantation the patient complained of blurred vision and ophthalmoscopy showed large globular bilateral inferior retinal detachments At this time the face was puffy but there was no peripheral oedema The detachments diminished very slowly but reattachment did not occur before death intervened

The postoperative course was characterized by persistent hypertension haematuria and proteinuria Four months after transplantation the patient died of a combined chronic allograft reaction and transplant glomerulonephritis This case has been described in detail by Petersen et al (6)

Case 4

O A (Fig 4) a 9 year-old man was transferred to this hospital in terminal uraemia secondary to chronic glomerulonephritis on August 5 1966 There was no history of acute nephritis In 1960 proteinuria and abnormal urinary sediment were incidentally discovered renal function and B P were normal He had felt well until July 11 1966 when he noticed puffiness around the eyes At the local hospital a serum creatinine of 8 3 mg/100 ml and a B P of 205/130 mm Hg were found

On admission to this hospital he complained of blurred vision and the optic fundi showed grade IV hypertensive changes with macular star figures retinal oedema and papilloedema Examination showed periorbital puffiness but no peripheral oedema

Treatment with intermittent peritoneal dialysis was instituted under which the general condition improved and the hypertension decreased. However uraemic intoxication was only partially controlled and the first haemodialysis had to be discontinued on account of pericardiac tamponade which was relieved after pericardiac puncture with evacuation of 1340 ml

Before the third peritoneal dialysis the patient was clinically overhydrated with pitting oedema over the sacrum and legs in spite of a weight loss of 2 kg since admission

After termination of the third peritoneal dialysis a routine ophthalmoscopy revealed two inferior globular retinal detachments on the right eye They remained unchanged at subsequent ophthalmoscopies until 30 days later when appreciable resolution had occurred and the

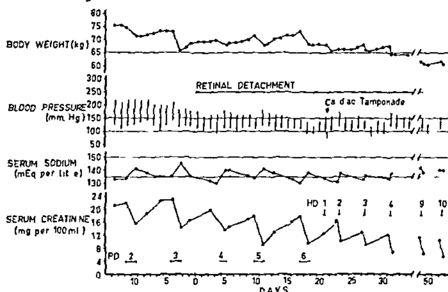


Fig 4 Case 4 PD = peritoneal dialysis HD = haemodialysis

vision had improved Twenty days later reattachment was complete and the hypertensive vascular changes in regression The patient later underwent su cessful renal allotransplantation and is now well

Case 5

A P (Fig 5) a 22 year old man was admitted to the local hospital in March 1966 complaining of symptoms of anaemia Hb was 6 9 g/100 ml and serum creatinine ? 4 mg 100 ml A renal biopsy showed a proliferative glomerular nephritis The renal function deteriorated rapidly and on transfer to this hospital on June 9 1966 the patient was oliguric and after a few days urine produ tion ceased completely Intermittent peritoneal dia lysis was instituted

On transfer B P and funduscopic examination were normal The B P increased slowly and two weeks later when it was 160/85 mm Hg streaky haemorrhages were observed in the right fundus Both fundi were oedematous but there were no vascular changes or papilloedema *Until the end of September ophthalmoscopy remained* unchanged

On October 14 when the patient had had hypertensive encephalopathy and episodes of convulsions and for two days had been comatose a routine funduscopic exa mination was performed This revealed pronounced ar teriolar narrowing haemorrhages and exudates The pa pillae were pale without oedema but the retinae were oedematous and on the right eye an inferior globular detachment was observed On October 25 the detachment

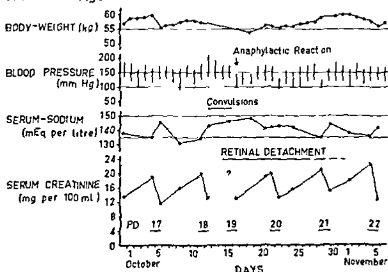


Fig 5 Case 5 PD = peritoneal dialysis. The anaphylactic reaction was due to serum infusion.

was markedly diminished and on November 3 reattachment was complete The retinal oedema was decreasing but the vascular lesions were unchanged.

There was no possibility for renal transplantation and the patient died on November 11 of a staphylococcal sepsis.

DISCUSSION

In retinal detachment a cleavage occurs in the potential space between the pigment epithelium which remains in position and the layer of rods and cones Retinal detachment may be due to several causes Commonly it is the result of a break in the retina which is usually located in the upper part of the eye this form is most frequently met with in elderly people and predisposed myopic persons Other ocular malformations may also be associated with retinal detachment and association with hereditary renal disease has been described (4)

The form of detachment dealt with here is the serous which is occasioned by accumulation of fluid in the subretinal space These separations are usually bilateral and potentially reversible Moore has described two types of retinal detachment occurring in renal retinopathy—the flat and the globular The globular is generally situated towards the periphery and involves the lower part of the retina as a result of gravitation the flat is most common over the central regions however he does not think that any essential pathological difference is indicated

In four of our patients the detachments were globular in one case 2 they were flat The patients were young the oldest aged 46 the ages of the others ranging between 14 and 29 years In none were there ocular malformations nor had retinal breaks been observed In three patients complete reattachment occurred within from nine days to seven weeks two patients died before reattachment but in both significant resolution had taken place In two patients the separations were unilateral only affecting the right eye

In the series of Lapco et al (2) the prominent feature was severe dilutional hyponatraemia In agreement with our series no correlation to the degree of azotaemia or hypoalbuminaemia was observed In contrast only two of our patients presented overt oedema at the time of detachment and one of them case 3 only in the form of periorbital puffiness Although subclinical overhydration was undoubtedly present in cases 1 and 2 universal fluid retention alone cannot explain the appearance of retinal detachment in our patients A brief episode of pronounced hyponatraemia occurred in case 1 and slight hyponatraemia was present in cases 3 and 5 before the detachments were observed However it seems quite unlikely that these small deviations alone could be of any importance

A joint feature in our series was hypertension with severe hypertensive vascular changes haemorrhages exudates and a very pronounced oedema of the retina However only three of the five patients had protrusion of the papilla In case 2 the disc margins were blurred and in case 5 the discs were normal Cerebrospinal fluid pressure was not measured but the appearance of the optic discs in the two last mentioned cases makes it unlikely that the explanation is to be found in a rise in this pressure

The clinical condition was characterized by hypertensive encephalopathy and uraemic intoxication

Like Lapco et al (2) we could demonstrate no consistent change in the level of the blood pressure before or after the onset of the retinal detachment nor after its reversal However as hypertension and severe hypertensive retinopathy were the only common factors in our series we feel that the retinal detachment must primarily be due to local retinal oedema caused by the hypertension and that fluid retention and uraemic intoxication were presumably contributory factors Hence the prophylaxis and therapy of retinal detachment in renal insufficiency consist in the prevention and treatment of these concomitants to renal failure

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INTRACARDIAC PHONOCARDIOGRAPHY IN THE DIAGNOSIS OF SMALI PATENT DUCTUS ARTERIOSUS WITH ATYPICAL MURMUR

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Abst act The diagnosis of a small patent ductus ar teriosus with minimal left to right shunt is made when the continuous murmur is recorded in the pulmonary artery by intracardiac phonocardiography during a simple right heart catheterization

The diagnosis was established in one patient in whom the patent ductus was the only lesion and in four pa tients with additional defects In none of the patients was the typ cal continuous murmur present at external auscultation two of the patients had only a systolic external murmur

The cardiac murmur in patients with a patent ductus arteriosus is often atypical in the presence of pulmonary hypertension (1 4 7) The con tinuous murmur may be replaced by a systolic murmur by no murmur at all or even by an isolated diastolic murmur (4)

Even with normal pressures in the pulmonary artery the murmur may be atypical (5) and the only auscultatory sign may be an isolated systolic murmur if the ductus is rather small (3)

It is the aim of this paper to demonstrate the diagnostic value of intracardiac phonocardiog raphy during right heart catheterization in pa tients with small patent ducti and atypical external murmurs in the presence of normal pressures in the pulmonary artery

METHODS

The Allard Laurens micromanometer—mounted on the tip of a no 8 double lumen card ac catheter—was used to record pressures and intracardiac phonocardiograms during a right heart catheterization (11) Blood samples were drawn for determ nation of oxygen saturation and in most patients an add tional hydrogen test was per formed (Table I)

DIAGNOSIS

In the presence of normal pressures in the pulmonary artery the blood flow from the aorta to the pulmonary artery throu h a patent ductus is constant throughout the cardiac cycle

The flow produces a continuous murmur When this murmur is recorded by the micromanometer placed in the pulmonary artery (Fig 1) the diagnosis is con sidered establ shed (3 5 8 10)

The murmur is most intense and of highest frequency when the t p of the catheter is situated just at the opening of the ductus and it is fainter as the tip is moved away from the point of origin In this way it is possible to distinguish between a patent ductus arteriosus with the maximum intensity of the murmur at the bi furcation of the main pulmonary artery and in the proximal part of the left pulmonary artery and an aorto-pulmonary window in which the site of greatest intensity of the murmur i more proximal in the main pulmonary artery ( )

The volume of the left to-right shunt through the ductus determines the transmission of the murmur within the pulmonary tree (? 6) If the shunt is of sufficient magnitude to be measured by determination of oxygen saturat on the murmur is usually present throughout the main pulmonary artery and its branches (still being most intense at the bifurcation) A murmur due to a smaller patent ductus is often only present in a limited area in the main pulmonary artery at the bifurcation close to the left pulmonary artery

MATERIAL AND RESULTS

From O tober 1964 to March 1967 the d agnosis of a small patent ductus arteriosus with a left to-right shunt undetectable by oxygen determination was considered establ shed in five patients by intracard ac phonocardiog raphy dur ng right heart catheterization (Table I) Four of the patients had additional defects In all patients a cont nuous murmur was recorded in the main pulmonary artery at the bifurcation (Fig 2)

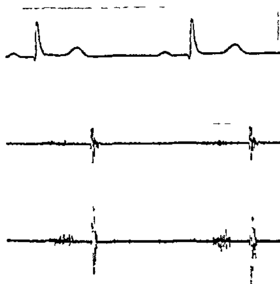
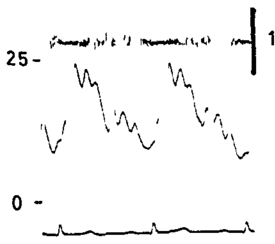


Fig 3 External phonocardiogram from the second left intercostal space in case 81 2 (Mingograph 31 B Elema Schonander)

detected but with the hydrogen electrode a small left to-r ght shunt to the pulmonary artery was demonstrated With intracardiac phonocardiography a continuous mur mur was recorded at a very restricted area in the main pulmonary artery just at the branching-off of the left pulmonary artery (Fig 4)

Auscultatory findings

At external auscultation a systolic murmur grade 2 or 3 (of 6) was heard in all patients the mur



F g 4 Intraca d phonocard ogram from the ma n pul monary artery n c se 81 (same patient as n Fig 3) The pres u e t ecorded through the s dehole

mur corresponded to the additional defects in four of the patients (Table I)

No typical continuous murmur was heard in any patient Two patients (cases 8122 and 7455) had only a systolic murmur while the remaining three patients also had a very faint diastolic murmur audible only just below the left clavicle In two of these patients (cases 1459 and 7360) the diastolic component was not heard until the auscultation was repeated after the heart catheterization

DISCUSSION

The diagnosis of a patent ductus arteriosus in the presence of normal pressures *in the pulmonary* artery is usually made clinically by the detection of the typical continuous murmur at the upper left sternal border and under the left clavicle

If the ductus is so small that only a negligible left to-right shunt is present the intracardia continuous murmur may be so faint that only the systolic component is transmitted clearly to the surface of the thorax while the diastolic com *ponent is either entirely lacking or barely au* ible at external auscultation

Such cases may easily remain undiagnosed the systolic murmur being interpreted as an innocent murmur Even if a small isolated patent arteriosus has no hemodynamic impl cause the minimal left to right shunt impose any strain on the heart, th still important because of the prop ures which have to be taken endocarditis

When hemodynamic studies diagnosis may be established heart catheterization with cardiography the continuo corded in the pulmonary in the patient with isolat series and this was also (3 5 9)

In the presence of the diagnosis may be heart catheterizat cardiac phonoca oxygen saturati hydrogen elect of the more

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THE LATE CARDIAC PROGNOSIS AFTER COXSACKIE B INFECTION

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Abstract Patients with positive Coxsackie B cultures but without diagnosed myocarditis were subjected to a cardiologic follow up examination after -11 years. At the time of follow up the men were at the most 35 years of age and the women were 45 years or less The possibility of cardiomyopathy was established in two of the 53 cases studied

The question often arises of whether a given case of myocardial disease of unclear etiology (cardiomyopathy) could be the result of an undiagnosed viral myocarditis (3 8) The majority of fatal cases of myocarditis elude clinical diagnosis (9) and the proportion of undiagnosed may be still greater for those which are not fatal A common cardiotropic virus is Coxsackie B (2 3) which may present clinically as pleurodynia myalgia aseptic meningitis pericarditis and myocarditis (3 7) More than one of these clinical manifestations are overt at times and a subclinical involvement of several organs may occur even more often Undiagnosed clinical involvement is conceivable especially in the case of myocarditis which is difficult to diagnose

The possibility of cardiomyopathy as a sequela of undiagnosed viral myocarditis could therefore be investigated by a follow up study of a group diagnosed virologically as having Coxsackie B infection with other clinical manifestations than myocarditis

MATERIAL

Initially all reports of positive Coxsackie B cultures (baby mouse or tissue culture—only occasionally confirmed serologically) from the National Bacteriological Laboratory Stockholm for the years 1953–1964 were examined From these were chosen for follow up all hospital treated men aged 18–35 in 1965 and women aged 18–45 who resided in or near the cities of Stockholm Eskilstuna Linkoping Vasterås Vaxjo and Örebro The group totaled 61 The upper age limits of 35 and 45 years were adopted to minimize the influence of coronary heart disease which seems to manifest itself approximately ten years earlier in men than in women (1) Only one of the 61 had been diagnosed as having a myocarditis and was excluded from the group Of the remaining 60 patients one was dead (retroperitoneal abscess) The other 59 were offered a cardiac examination and 53 (90) appeared 30 being men between 18 and 35 years (mean 27) and 23 women between 18 and 45 years (mean 31) Eighteen patients resided in Stockholm and the remainder in other areas of Sweden The primary clinical symptom at the time of treatment had been meningitis in 33 cases encephalitis in three pleurodynia in six pericarditis in two and nonspecific complaints (usually fever pharyngitis severe headache) in nine The virus had been typed as B 5 in 6 patients B 4 in 13 B 2 in five B 1 in three and B 3 in one while the type number was lacking in five cases The interval since infection varied between 2 and 11 years with a mean of six years

METHODS

At follow up in 1965 the patients were asked about cardiac symptoms before and after hospitalization the points of enquiry being heart failure (dyspnea on walking paroxysmal nocturnal dyspnea and dyspnea at rest) effort angina (effort or excitement produced chest pain necessitating relative rest and lasting no more than 15 minutes) arrhythmias (attacks of very rapid heart rate with sudden onset periodic slow pulse or syncopal episodes) and possible cardiac medications In the physical examination special attention was paid to respiratory difficulty at rest lip cyanosis neck vein distention ankle edema and liver enlargement The heart and lungs were auscultated and blood pressure was measured An ECG at rest was taken with a 4 or 3-channel ink writing machine (Mingograf Elema Schonander AB Stockholm) Leads I II III aVR, aVL aVF V V and V were always recorded and usually also V_2 V_4 CR CR, CR, CR, and CR The paper speed was 50 mm/sec The ECG was interpreted according to the criteria in (4) The radiological heart volume was calculated from chest films (frontal and side views) (5) The upper limit of normal was considered to be 500 ml/m body surface

Table I *Symptoms and findings*

Sex	Age	Symptoms	Signs	ECG
♀	35	PND	P 2	AV block I
♀	35	PND RD		Nodal rhythm
♂	31	ED	P 2	
♀	22	ED		
♀	36	PND		
♀	32	RD		
♀	21	PT		
♂	33		C	
♀	31			Pre-excitation

ED = Effort dyspnea PND = Paroxysmal nocturnal dyspnea RD = Dyspnea at rest PT = Paroxysmal tachycardia C = Cyanosis of the lips P 2 = Accentuated pulmonary second sound

for men and 450 ml/m for women (6) All films showing a heart volume near or above the upper limit of normal were reviewed by one radiologist

RESULTS

Of the 53 patients studied ten had cardiologic symptoms or findings which were clearly or possibly pathologic See Table I All of these symptoms or findings seem to have developed after the Coxsackie B infection No ECGs from the time of infection or earlier were available for those cases with ECG changes at follow up

One case with dyspnea associated with other signs of pulmonary disease has been omitted from the table as has a case with dyspnea associated with marked obesity Heart volumes were normal in both of these cases as in all others in the study

DISCUSSION

For the investigation of the role of a common disease like Coxsackie infection in the causation of an uncommon entity like cardiomyopathy one must have a large study group It is not possible to determine the exact size necessary since the incidence of the diseases is not known and it may be that the present group is too small for the purpose

In the absence of acute and convalescent serum antibody titers it cannot be assumed that an acute Coxsackie B infection has been established in every one of these cases but the group should provide a high concentration of patients with previous infections

A combination of symptoms occurred in two cases A 35 year-old woman had paroxysmal nocturnal dyspnea a strongly accentuated second sound over the second left interspace and first degree AV block (PR = 0 21 sec at 80/min) Another 35 year-old woman had paroxysmal nocturnal dyspnea intermittent dyspnea at rest and a nodal rhythm In addition both of these women were among the three patients who had heart volumes near the upper limit of normal (410 and 450 ml/m) This combination of unusual symptoms provides grounds for suspecting cardiomyopathy At the time of hospitalization 4–5 years earlier physical examination had been normal in both cases ECG and chest X ray had not been made in either of the cases The infection had manifested itself as meningitis in both cases The virus types had been B 5 and B 2 respectively

An additional four patients complained of dyspnea in one form or another and there was an accompanying accentuation of the second sound over the second left interspace In the absence of cardiac enlargement and ECG changes in these cases the dyspnea cannot be said to be secondary to myocardial disease Paroxysmal tachycardia in one case and cyanosis in another had developed after hospitalization but cannot be attributed to Coxsackie B infection on that basis alone Furthermore it is possible that the pre excitation in one case was present in the normalized form at the time of hospitalization

A finding worthy of notice is the presence of a short PR interval in as many as four cases 0 10 sec in a 17 year-old man and 0 11 sec in a 45 year old woman as well as 29 and 22 year old men These four had otherwise normal findings on examination except for bradycardia in one and polio related scoliosis in another None had an ECG from the time of hospitalization As a control 500 ECGs were reviewed from health examinations on civil servants of corresponding ages Only one case from that group a 27 year old woman was identified with a PR interval of 0 11 sec or less ($P < 0\ 05$) Even if a cause and effect relationship should lie behind this statistical connection between Coxsackie B infection and a short PR interval it does not necessarily signify cardiac damage since the AV node is under hormonal and nervous influence

The two cases with pericarditis had no symptoms or findings at follow up

In conclusion among these 53 cases two were found with an accumulation of cardiologic symptoms and findings which were otherwise uncommon in this study The symptoms and physical findings seem to have appeared after Coxsackie B infection No comparative material is available for the ECGs It therefore seems possible that cardiomyopathy is present in both of these cases

ACKNOWLEDGEMENT

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THE LATE CARDIAC PROGNOSIS AFTER ACUTE CARBON MONOXIDE INTOXICATION

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Abstract A follow up study of 23 hospitalized cases of acute carbon monoxide poisoning showed –23 years after the event, ECG changes in five cases to be the only abnormal cardiologic finding

Chronic myocardial disease of uncertain etiology need not represent an unknown disease It can also be considered to be a late stage of known disease or intoxication With respect to intoxications long term alcohol misuse is thought to be able to give rise to a cardiac complication the so-called "alcoholic heart disease Another poison which can effect the heart is carbon monoxide Chronic poisoning in the form of long standing occupational exposure however seems seldom or never to lead to cardiac damage as judged by an ECG study (8) At least one case of chronic myocardial disease on the other hand has been described after acute carbon monoxide poisoning (11) That involved a 35 year old man who had been free of cardiac disease until he was exposed to carbon monoxide poisoning Two years after the exposure he still had chest pains dyspnea and T inversion on the ECG

In order to get an impression of the incidence of lasting cardiac damage after acute carbon monoxide poisoning a follow up study was done on a group with that diagnosis

MATERIAL

All available charts with the diagnosis of acute carbon monoxide poisoning from the years 1940–1964 were studied at twelve of Stockholm s fourteen departments of medicine It was unusual for the charts to contain information on COHb determinations and where data were given the intervals betw [illegible] termination of exposure and taking of the sample were not indicated All males over 35 and women over 45 were excluded from the follow up study in order to minimize the influence of coronary heart disease which seems to manifest itself approximately ten years later in women than in men (1) The remaining charts were reviewed with reference to support for the diagnosis acceptance into this study requiring either a COHb concentration of at least 10 or in the absence thereof the odor of gas or smoke at the place where the patient became sick coupled with unconsciousness which disappeared en route to the hospital or shortly after arrival there Forty two cases were obtained fitting these criteria and all except three could be traced Of the thirty nine cases traced four were dead four had moved to another country and five lived in rural Sweden The 6 patients who lived in Stockholm were invited for cardiac examination and 73 (88%) presented themselves Two failed to appear for unknown reasons and the third was unable to come because of cancer

Of the twenty three in the follow up study eight were men of 23–35 years (mean 8) and fifteen women of 25–45 years (mean 40) The diagnosis in seven cases was made on the basis of a *COHb* concentration of *10–46* and in sixteen cases on the basis of gas or smoke odor at the place of onset with temporary loss of consciousness In at least twelve cases there was a question of attempted suicide

METHODS

At follow up in 1965 the patients were asked about cardiac symptoms before and after the carbon monoxide exposure the points of enquiry being heart failure (dyspnea on walking paroxysmal nocturnal dyspnea or dyspnea at rest) effort angina (chest pain related to exertion or excitement necessitating relative rest and lasting 15 minutes at the most) arrhythmias (attacks of very rapid heart rate with sudden onset periodic slow pulse or episodes of syncope) and possible cardiac medications In the physical examination dyspnea at rest lip cyanosis neck vein distention leg edema and hepatic enlargement were sought the heart and lungs were auscultated and the blood pressure was measured An ECG at rest was taken with a four-channel ink writing machine (Mingograf 4_ Elema Schonander AB Stockholm)

Leads I II III AVR AVL AVF CR , , V were recorded at a paper speed of 50 mm/sec The ECG was interpreted according to the criteria in (3) *The roentgenological heart volume was calculated* from chest films (frontal and side views) (7) The upper limit of normal was considered to be 500 ml/m² body surface area in men and 450 ml/m² in women (9)

RESULTS

Of the four dead none had heart disease diag nosed on the death certificate

Of the twenty four followed up three patients gave a history of dyspnea on walking which arose after carbon monoxide intoxication All were women in their 40 s None had any evidence of incompensation on physical examination and none had any radiologic cardiac enlargement Ef fort angina was *denied* by all *patients* One pa tient gave a history of paroxysmal tachycardia which had begun since the intoxication episode All denied syncopal attacks with effort and in the *sitting or lying position No sign of heart failure* was present upon physical examination It was considered that early systolic medium pitched heart murmurs were insignificant if they were of *maximum intensity along* the left sternal border and were grade /6 or less No other type of murmur was found in any case except for one 23 year old man who had an early systolic *medium pitched murmur of grade /6 which* was heard best over the first right interspace and was accompanied by a normal second sound no ECG changes and normal heart volume

The ECGs showed chiefly changes in rhythm Of two brothers exposed simultaneously at the ages of eight and six one now (21 years later) had a first degree AV block (PR 0 24 sec) and *the other had a sinus bradycardia (48/min)* A 42 year-old woman had a regular supraventricular rhythm from a focus other than the SA node (P_{II} and P_{III} were negative and PR 0 11 sec) *Another 42 year-old* woman *with no sign* of myxedema had low voltage (QRS amplitude <0 5 mV in the limb leads and <1 0 in the chest leads) Depression (¹/ mm) of the ST junc *tion with horizontal* ST segments and low am plitude T waves in V (1 mm) occurred in one 44-year-old woman In each of these cases the ECG changes were the only finding of the follow *up investigation*

DISCUSSION

Exertional dyspnea in the absence of cardiac *enlargement cannot be attributed to cardiac* disease Paroxysmal tachycardia had developed in one case a few years after carbon monoxide ex posure but it cannot from that fact alone be *said to be due to intoxication Apart from one* case with a heart murmur which was difficult to interpret there were no abnormal findings in the physical examination and chest X rays

The four cases with AV block ectopic atrial rhythm low voltage and ST T changes could have been the result of carbon monoxide poison ing since similar changes have been described in *connection with acute poisonings (5 10) Upon* comparison of ECGs it was shown that the low voltage changes had arisen after the intoxication In the other three cases comparison ECGs are *lacking and the changes cannot be said with* certainty to have developed after the carbon monoxide exposure First degree AV block like low voltage has been described in cardiomyop*athy (6) but chief symptoms such as heart* failure Stokes Adams attacks and cardiomegaly are missing in all of the cases with ECG changes

Among the cases with high COHb values (30–47 %) *there was only one with cardiac symptoms* or findings that being the case with low voltage on the ECG

There is thus no adequate basis for the diag *nosis of cardiomyopathy in any of these 23 cas s* The group is small but it is obvious that cardio myopathy is not a common development even after severe acute carbon monoxide poisoning

ACKNOWLEDGEMENT

This investigation has been supported by the Swedish National Association against *Heart and Chest Diseases*

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THE LATE CARDIAC PROGNOSIS AFTER NON PENETRATING CHEST TRAUMA

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Abstract Fourteen patients with radiologically diagnosed sternal fractures have been studied after / to 16 years for the possibility of persistent cardiac damage In no case were there cardiac findings which could be definitely attributed to the non penetrating chest trauma

In the presence of a case of chronic cardiac diseas of unclear origin the question occasionally arises of whether an earlier chest trauma could have played an etiological role In a study of 546 autopsy cases of cardiac injury from non penetrating chest trauma which was not the direct cause of death Parmley et al found that contusion laceration was the most common occurrence but that rupture of the atrial septum ventricular septum, papillary muscles chordae tendinae or h art valves could also result as could hemopericardium (6) These injuries may cause heart failure and angina pectoris in the acute course (2 6 7) but it seems doubtful whether the symptoms can becom permanent (6) As lasting sequelae on the other hand arrhythmias as well as murmurs ECG changes and cardiac enlargement have been reported

In order to get an impression of the incidence of lasting cardiac symptoms in patients with previous non penetrating cardiac injury a follow up study was done on a group with sternal fractures

MATERIAL

From all patients diagnosed as having sternal fractures by the radiology department of the Serafimer hospital dur ng the years 1949 to 1964 those were select d who had been li ing in Stockholm at the tim of the injury and who at the time of follow-up were at or below the age of 35 for men and 45 for women These age l mits were used in order to min mize the influence of coronary heart disease which seems to manifest its f approximately ten years later in women (1) The names of 16 patients were obtained in this way and all could be traced through the population registry and were invited for follow up study Fourteen patients appeared for the examination (88%) of which ten were men aged 21 to 35 and four women aged 25 to 43 The time since injury was / to 16 years with a mean of seven years The sternal fracture had resulted in twelve cases from a direct trauma usually during an automobile accident and had been caused by indirect trauma in two

METHODS

The examination included history physical examination, ECG and screening X rays of the heart and lungs The history was taken with standardised questions and was concerned with heart failure (dyspnea on walking paroxysmal nocturnal dyspnea and dyspnea at rest) angina pectoris (effort or excitement produced chest pain necessitating relati e rest and lasting 15 minutes or less) arrhythmias (attacks of rapid heart rate with sudden onset periodic slow pulse or syncopal episodes) and cardiac medication. The physical examination included general condition (resp ratory distress cyanosis neck veins, and edema) heart, blood pressure lungs and liver Early systolic m d um pitched murmurs which were heard best along the left sternal border and were of grade 2/6 or less were o erlooked as being probably funct onal The ECGs were taken with a 4-channel ink writing machine (Mingograf 42, Elema Schonander AB Stockholm) Leads I II III aVR, aVL aVF CR CR_2, CR CR V V_2, V_4, V_6 and V were recorded at a paper speed of 50 mm sec The ECGs were then e aluated as by Goldman (3) Heart volume per square meter body surface was calculated as in (4) from the screening films (frontal and side views) In men 500 ml m body surface area was considered to be the upper limit of normal and in women 450 ml m (5)

RESULTS

Ea h of the 14 patients gave negative replies to all questions on heart failure angina pectoris and arrhythmias The physical examination turned out

to be normal in all cases except that of a 34 year old man He had a high pitched early systolic murmur grade 3/6 which was heard best at the fourth left interspace and transmitted to the apex as well as to the second right interspace The second sound at the second right interspace was normal Blood pressure was 150/85 The ECG showed a shortened PR interval 0 11 sec but was otherwise normal and films of the heart showed no cardiac enlargement or abnormal configuration In all other patients the ECGs as well as heart lung X rays were normal

DISCUSSION

The murmur in the 34 year-old man was not typical of any of the cardiac defects which have been reported to develop after non penetrating chest trauma (aortic insufficiency mitral insufficiency and ventricular septal defect) (6) In the absence of additional symptoms further cardiac investigation was not felt to be indicated

No definite case of permanent cardiac damage could therefore be demonstrated among these 14 cases The group is small but it is apparent that lasting cardiac sequelae are not common after non penetrating chest trauma

ACKNOWLEDGEMENT

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THE FREQUENCY OF THROMBOCYTOPENIA IN PATIENTS WITH HEART DISEASE TREATED WITH ORAL DIURETICS

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Abstract The occurrence of asymptomatic thrombocytopenia in patients with heart disease during treatment with oral diuretics has been investigated The study is retrospective and comprises an analysis of 244 consecutive cases admitted to a cardiological ward during a period of six months A platelet count below 100×10^3/mm was regarded as indicative of thrombocytopenia In the control group (93 subjects) four cases and in the diuretic group (71 subjects) 19 cases with thrombocytopenia were found Of the remaining 80 subjects seven had thrombocytopenia six of whom had had oral diuretics

Concomitant administration of other drugs such as quinidine or digitoxin could not account for the difference between the control group and the group treated with oral diuretics In the diuretic group right heart failure and enlargement of the heart was however more prevalent It is concluded that in patient with heart failure there is a considerable risk of the development of thrombocytopenia during treatment with oral diuretics

Oral diuretics have been extensively used during the past ten years Since the introduction of chlorothiazide and related diuretic agents scattered reports of blood dyscrasias due to the administration of these drugs have been published Most of the reported cases had thrombocytopenic purpura A few had thrombocytopenia without purpura (2 3 5 8 10 18 21 23 28) and a limited number of cases had purpura without thrombocytopenia (7 9 12 14) Cases of agranulocytosis and neutropenia (6 8 13 16 25 28) were also reported Not all of the reported cases however fulfil Ackroyd s (1) criteria for a valid demonstration of a drug induced thrombocytopenia

Considering the wide use of oral diuretics and the paucity of reported thrombocytopenias caused by these drugs one would tend to regard the occurrence of this condition as rare This is also the opinion of the A M A Council on Drugs (20) which states blood dyscrasias have been reported infrequently

We have however fairly often encountered patients manifesting asymptomatic thrombocytopenia during administration of oral diuretics It was therefore considered to be of clinical importance to study the frequency of thrombocytopenia in patients treated with these drugs

MATERIAL AND METHODS

The records of 304 patients admitted to a cardiological ward during the first six months of 1964 were reviewed A platelet count was performed on 244 patients during that period and those were chosen for investigation The study is retrospective and none of us was associated with the ward We have chosen this unit for study because diuretics were frequently used and platelet counts almost routinely performed on all patients admitted to this ward

The material was divided into three groups as follows

I *Control subjects* who for at least two months prior to admission did not have oral diuretics The group comprises 93 subjects 54 men and 39 women The age ranged from 16 to 82 with an average of 44 years

II *Subjects treated with oral diuretics* (diuretic group) The group comprises 71 subjects 28 men and 43 women The ages ranged from 29 to 77 years with an average of 56 years

III *Remaining subjects* These patients could not be placed in either of the above groups because they had diseases which are likely to give a pathological platelet count e g cirrhosis of the liver systemic autoimmune diseases myeloproliferative diseases malignant tumors thrombotic states and acute blood loss Patients who were on steroid therapy and finally patients whose drug therapy prior to admission was not stated in the record were also placed in this group The group comprises 80 subjects 4? men and 38 women The ages ranged from 15 to 9_ years with an average of 53 years

The composition of the three groups with regard to diagnosis is given in Table I

Platelet counts were made by the technique of Kristenson (17) Although the normal range at this laboratory is 150-350 000 mm we regarded a platelet count below 100 000 mm as thrombocytopenia

Table 1 *The distribution of the material with respect to diagnosis in the three groups studied*

	No of cases		
	Control group	Diuretic group	Others
Congenital heart disease	16	2	4
Rheumatic valvular disease	38	44	21
Syphilitic aortitis		1	
Arteriosclerotic heart disease	11	12	20
Acute myocardial infarction	3	2	1
Cardiomyopathies	2	1	1
Arythmias	13	4	3
Other heart disease	2		5
Hypertension		3	
Cerebral vasc Thromb	1		1
Pneumonia	1	1	
Rheumatoid arthritis		1	
Liver disease			9
Miscellaneous			15
Observation	6		

The records were critically reviewed. All drugs ad min stered during the period of study were tabulated and their causal relationship to the pathological platelet count analyzed The patients were classified with regard to left sided and right sided heart failure The volume of the heart was estimated by X ray according to the method of Jonsell (15)

RESULTS

The age distribution and the mean platelet count of the three groups and the mean platelet count in every age class of each group are shown in Fig 1 The mean platelet count of the group who received oral diuretics was lower than in the two other groups but not significantly below the mean of the control group There was no significant difference between the mean platelet count of the different age classes within each of the groups

The individual platelet counts are shown in Figs 2 and 3 The symbols represent a mean value if several counts were made or the lowest value if a gradual fall of the platelet count with the development of thrombocytopenia occurred during the time of observation Since quinidine and digitoxin are involved in drug induced thrombocytopenia (4 11 19 24 27) the subjects who received these drugs have been indicated in Fig 2

1 The control group (93 subjects)

Twenty six subjects had digitoxin 17 digitoxin and quinidine and five quinidine alone For the whole group 128 platelet counts were performed The mean platelet count for the group was 171 ×

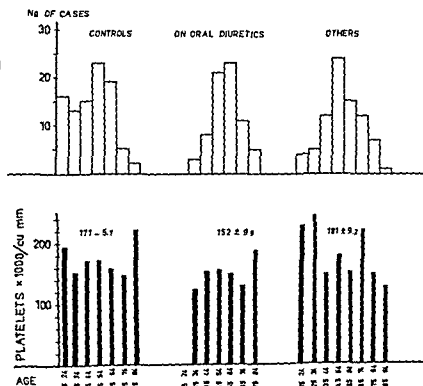


Fig 1 Age distribution of the three groups studied The loaded columns indicate the mean platelet count of the respective age group The figures give the mean and s.e for the whole group

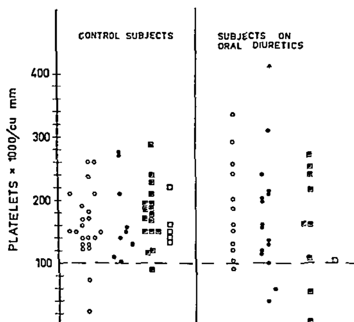


Fig 2 The individual platelet counts in the control and diuretic group Subjects on digitoxin (●) quinidine (□) digitoxin and quinidine (◙)

$10^3 \pm 5.7/mm^3$ Four subjects (4) had thrombocytopenia One of them had a mean count of $23 \times 10^3/mm^3$ the others only a moderate diminution of the platelet count (Fig 2) Two of the thrombocytopenic subjects had no drugs one had digitoxin and one quinidine The cause of thrombocytopenia in these subjects could not be elicited from the records

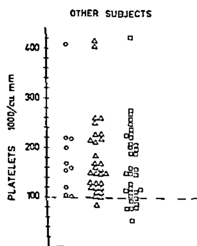


Fig 3 The individual platelet counts in the others. Subjects who did not have oral diuretics (O) on oral diuretics (□) uncertain if they had diuretics before admission (Δ)

II *The group on oral diuretics* (71 subjects)

Forty eight subjects of the group had digitoxin nine digitoxin and quinidine and one quinidine alone The type of oral diuretic used is given in Table II For the whole group 297 platelet counts were performed The mean platelet count for the group was $152 \times 10^3 \pm 9.9/mm^3$ The individual platelet counts are shown in Fig 2

Nineteen subjects nine men and ten women aged 42 to 72 with a mean age of 56 years had thrombocytopenia (26 % of the group) Again the type of the diuretic agent given to the throm

Table II *The type of the oral diuretic administered to the respective groups and to the thrombocytopenic subjects*

	No of cases			
	Diuretic group		Others	
	Total	Thrombo-cytopenic	Total	Thrombo cytopenic
Chlorthalidon	52	14	21	4
Chlorothiazide	7	3	3	
Hydrochlorothiazide	6	1	4	1
Bendroflumeth azide	3	1	5	
Trichlorometh az de	2			
Polyth azide	1			
Chlorthal don – hydrochlorothiazide			1	1

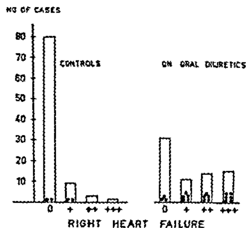


Fig 4 The distribution of the degree of right heart failure Dots indicate the thrombocytopenic subjects

bocytopenic subjects is also shown in Table II Sixteen were treated with digitoxin and two with digitoxin and quinidine (Fig 2) Their platelet count varied from 8000 to 93 000 with an average of 53 000/mm³

An analysis of the thrombocytopenic patients showed that in seven cases the platelet count had risen rapidly after withdrawal of the oral diuretic although no other changes in the patients treatment had been made In another seven cases the thrombocytopenia was probably due to the oral diuretic but evidence of the causal relationship was lacking since platelet counts were not made after withdrawal of the drug In some of the last subjects thrombocytopenia due to an oral diuretic could be demonstrated during other periods of admission In the remaining five cases the cause of the thrombocytopenia was impossible to evaluate since there were other circumstances such as infection or congestion of the liver which could equally well have caused thrombocytopenia

The sensitivity of the above 19 subjects to diuretics was also studied during other periods as far as records were available Some of them showed a thrombocytopenia whenever exposed to the offending drug and their platelet count rose when the oral diuretic was withdrawn Others were on some occasions severely thrombocytopenic but after improvement of cardiac function e g postcomissurotomy their platelet count was found to be within the normal range in spite of the administration of the same diuretic agent

A comparison of the control group with the group treated with oral diuretics in respect of the degree of right sided heart failure and of heart volume as determined by X ray has shown that decompensation and enlargement of the heart were more usual in this latter group (Figs 4 and 5)

III *Others* (80 subjects)

Thirty four had oral diuretics (Table II) Thirty six had digitoxin six digitoxin and quinidine and two quinidine The mean platelet count for the whole group was $181 \times 10^3 \pm 9.2/mm^3$ The individual platelet counts are shown in Fig 3 The subjects were divided into three subgroups those who had no diuretics those whose records prior to admission failed to indicate whether they had had diuretic therapy and finally those who were on oral diuretics It is seen from the figure that thrombocytopenia was present in seven subjects and that six of them had oral diuretics In one case there was evidence of chlorthalidone induced thrombocytopenia in two chlorthalidone could have been the causative agent while in the rest hepatic or malignant disease was probably the cause

COMMENT

Blood dyscrasias are commonly regarded as an unusual complication to treatment with modern diuretics The present study however has shown that 26 % of subjects in the diuretic group had thrombocytopenia but only 4 % in the control group Concomitant administration of quinidine known to cause thrombocytopenia in sensitive subjects could not be responsible for the high in

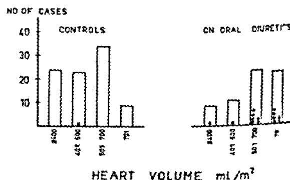


Fig 5 Heart volume determined by teleroentgenography and expressed in ml per m² body surface in the groups studied Dots represent the thrombocytopenic subjects

cidence of thrombocytopenia in the diuretic group since a greater perc ntage of subjects was given quinidine in the control group and only two thrombocytopenics in the diuretic group had quinidine Thrombocytopenia due to digitoxin is extremely rare and only three cases have hitherto been reported in the literature since the first re cognition of the condition in 1952 (4 19 27)

The control group may not be comparable with the diuretic group because of a greater degree of cardiac failure and overall illness in the latter which per se could be responsible for the high frequency of thrombocytopenia in this group There are however strong indications of a causal relationship between the treatment with diuretics and thrombocytopenia in the majority of cases It could be demonstrated that the platelet count returned to normal after withdrawal of the drug in seven cases and in another seven cases the causal relationship between the diuretic agent and the thrombocytopenia was highly suggestive

The high incidence of asymptomatic thrombo cytopenia in patients with heart disease receiving oral diuretics encountered in this retrospective study confirms our clinical impression that throm bocytopenia is not seldom a complication to therapy with these drugs (26) The paucity of reported thrombocytopenias caused by oral diuret ics (20) which contrasts with the results of the present study may have several explanations One of th m is the possibility that cases without mani fest purpura are seldom recognized and the sec ond that the number of reported cases represent only the tip of the iceberg

The mechanism by which thiazides and related compounds induce thrombocytopenia is not clearly understood Most authors could not de monstrate platelet antibodies in the sera of pa tients suffering from thiazide induced thrombo cytopenic purpura Thrombocytopenia on the other hand can be explained by an incompetent bone marrow This can be caused by drugs that are uniformly toxic to the bone marrow of all persons if a sufficient concentration of the agent is present or by drugs that apparently affect only the bone marrow of sensitive subjects Hypo plastic marrow and decreased megakaryocyte production was observed in several studies (22 28) Provocative administration of thiazides to patients who had thrombocytopenia due to the drug induced a gradual platelet reduction not until after 5 to 10 days exposure (26) These ob servations as well as the large number of thrombo cytopenias found in the present study are in favor of a myelosuppressive action rather than an im munological mechanism If the mode of action is related to toxicity there might be a correlation between dosage duration of treatment and throm bocytopenia This point however could not be elucidated from the present study

It is concluded that in patients with heart failure there is a considerable risk of the d velopment of thrombocytopenia during treatment with oral diuretics The possibility of this complication might be of special importance when the patient is on anticoagulant therapy or when there is concomitant liver disease

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IRON FORTIFIED BREAD

Absorption and Utili ation Studies

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Abstract The increase in serum iron two hours after test meals in fasting subjects was taken as an expression of iron absorption in three series of experiments All the ind viduals most of them young healthy female nurses acted as their own controls Tests were performed with white wheaten and wholemeal bread fortified with ferrous sulphate or ferrum reductum to 40 mg iron per 100 g. Some experiments with unenriched white bread were also included

It was found that ferrous sulphate gave a more pronounced increase in serum iron than ferrum reductum The increase was higher after fortified white bread than after fortified wholemeal bread This was probably due to the inhibitory effect, although only partial of dietary phytate on iron absorption

B cause of the relative uncertainty of the performed absorption experiments a controlled clinical trial with ferrous-sulphate-enriched white and wholemeal bread to 8 mg iron per 100 g was conducted in 146 adult female patients of a mental hospital The 12 weeks trial was completed in 77 subjects in the experimental group and in only 0 in the control group

In the experimental group the mean Hb value showed a significant increase to 14 32 g % which is equivalent to the mean optimal value for adult Norwegian women. A concomitant rise in the mean Hct and MCHC values was recorded The results in the control group are dif f cult to interpret The mean increase in Hb concentra t on after 1 weeks, however was not statistically signif icant.

The present study indicates that ferrous sulphate baked into bread is better absorbed than ferrum reductum and is easily a ailable for hemoglobin synthesis

A long term community based feeding trial, however may be necessary before a final conclus on regarding the future Norwegian iron fortification policy is reached

Recent studies of Norwegian population groups (9 11 12 13 14 21) have revealed a rather high prevalence of iron-deficiency anemia among schoolchildren adolescent grammar school pupils young men women in the reproductive age middle aged men and old people In addition it has been disclosed by dietary surveys that the ordinary Norwegian diet is relatively low in iron and that many individuals get considerably less iron than recommended (2 3 15 16 19 20 24) This is why the question of iron enrichment is of present interest in Norway

Since 1939 fortification of certain staple foods with vitamins and minerals has been encouraged in many countries in particular the addition of thiamine riboflavin niacin calcium and iron to white flour and white bread The enrichment programmes have either been performed on a voluntary basis as in Belgium Canada Holland Italy Switzerland and Sweden or made compulsory as in D nmark Chile Great Britain Newfoundland and 27 of the states of the USA (23)

Assessment of the absorption and utilization of iron from bread made with fortified flour however has been difficult due to discrepancies in the results reported by different authors (4 8 10 17 18 22)

Bread fortified with ferrous carbonate (10) or ferrum reductum (4 22) had no appreciable effect on hemoglobin (Hb) levels in children and female patients of a mental hospital Radioactive studies (17) balance experiments (8) as well as hematological studies (18) however have revealed that ferrous sulphate ferrum reductum as well as various ferric compounds added to bread are a significant source of iron in human nutrition

Due to the present confusion and controversy regarding the effect of iron fortified bread a national enrichment scheme has not yet been proposed to the authorities The prevalence of iron

Table I *Preliminary trials (experiment I) The serum iron concentrations before and two hours after the ingestion of 100 g white wheaten bread unenriched or enriched with ferrous sulphate or ferrum reductum to 40 mg iron per 100 g*

Subjects			Serum iron concentration (µg Fe/100 ml)								
			Ferrous sulphate			Ferrum reductum			Unenriched		
Age (y)	Sex	Clinical diagnosis	Before	2 h after	Increase	Before	2 h after	Increase	Before	2 h after	Increase
42	o	Healthy	114	121	7	14?	150	8	132	131	(−)1
4_	o	Posthemorrhagic anemia	30	75	45	36	59	23	39	38	(−)1
30	o	Healthy	71	90	19	104	112	8	118	1 0	
31	o	Healthy	128	142	14	161	198	37	79	103	?4
50	o	Myocardial infarction	61	66	5	46	50	4	60	63	3
33	o	Healthy	104	95	(−)9	126	112	(−)14	136	122	(−)14
36	♂	Healthy	79	86	7	99	109	10	7?	79	7
69	♀	Myocardial infarction	57	50	(−)7	41	34	(−)7	56	54	(−)
32	o	Healthy				103	101	(−)2	104	91	(−)13
30	o	Healthy	141	151	10	99	114	15			
66	o	Heart failure	97	93	(−)4	85	81	(−)4			
48	o	Myocardial infarction	85	112	27	83	71	(−)12			
64	♂	Pericarditis	105	120	15	139	154	15			
59	o	Myocardial infarction	63	54	(−)9	114	114	0			
32	o	Pneumonia	116	119	3	135	116	(−)19			
48	♀	Healthy	79	149	70	_9	32	3			
Mean			89	102	13	96	100	4	88	89	1

deficiency in Norway however makes the desirability of such a programme apparent should it be possible to find an iron compound which is readily available to man when added to flour

This paper describes two sets of experiments designed to evaluate the absorption of two iron compounds and the utilization of iron when given as ferrous sulphate in bread

A ABSORPTION STUDIES

Three series of experiments (I II and III) were made and the serum iron increase two hours after the ingestion of a test meal with iron fortified bread was used as an expression of iron absorption

Material and Methods

A preliminary trial was made in 16 individuals (experiment I) Table I shows the sex age and clinical diagnosis of these subjects Healthy female nurses aged 0 to 30 years participated in the next two series of experiments, 18 individuals in experiment II (Table II) and _0 in experiment III (Table III)

One hundred g of the fortified bread was eaten fasting in the morning together with 00 ml distilled water Blood specimens were drawn before and two hours after

Table II *Experiment II The serum iron concentrations in 18 young healthy women before and two hours after the ingestion of 100 g white wheaten bread enriched with ferrous sulphate or ferrum reductum to 40 mg iron per 100 g*

Subject no	Serum iron concentration (µg Fe 100 ml)					
	Ferrous sulphate			Ferrum reductum		
	Before	2 h after	Increase	Before	2 h after	Increase
1	40	57	17	51	86	35
2	61	66	5	68	69	1
3	65	99	34	71	87	16
4	36	95	59	43	79	36
5	104	149	45	135	1 0 (	)15
6	66	133	67	55	74	19
7	6?	114	5_	97	1_4	7
8	58	83	?5	95	110	15
9	140	195	55	49	58	9
10	49	74	_5	1??	138	16
11	77	1?4	47	86	99	13
12	81	123	4?	193	165 (	) 5
13	48	96	48	79	10	3
14	86	11_	6	74	80	6
15	8	89	61	35	45	13
16	54	88	34	70	8	)
17	94	111	17	78	90	)
18	68	107	39	79	86	7
Mean	65	106	38	8_	94	?

Table III *Experiment III The serum iron concentrations in 20 young healthy women before and two hours after the ingestion of 100 g white wheaten or wholemeal bread enriched with ferrous sulphate to approximately 30 mg iron per 100 g*

	Serum iron concentration (μg Fe/100 ml)					
	White wheaten bread			Wholemeal bread		
Subject no	Before	2 h after	Increase	Before	2 h after	Increase
1	58	77	19	49	53	4
2	71	99	28	105	140	35
3	90	114	24	105	105	0
4	32	118	86	34	49	15
5	65	132	67	195	223	28
6	54	75	21	93	122	29
7	96	119	23	122	142	20
8	7	97	40	64	104	40
9	103	147	44	90	126	36
10	93	304	211	72	168	96
11	179	314	135	63	102	39
12	103	173	70	160	187	27
13	144	179	35	159	176	17
14	88	147	59	88	119	31
15	105	199	94	151	170	19
16	129	160	31	179	185	6
17	80	120	40	133	165	32
18	99	171	72	112	198	86
19	98	159	61	144	173	29
20	109	126	17	136	140	4
Mean	93	152	59	113	143	30

the test meal and the serum iron concentration was determined by the Teepolbathophenanthroline method modified by Askevold and Vellar (1)

Two or three experiments employing different specimens of fortified bread were conducted on all the subjects in order to let each individual act as his own control. The experiments were carried out two or three days in succession and the sequence of the different kinds of bread was randomly chosen

The fortified bread was baked under the supervision of Dr Arne Schulerud at the experimental bakery of the State Technological Institute Oslo Formulas typical of the Norwegian white bread (loff) made of 78% extraction wheat flour and of wholemeal bread (Kneipp-brød) made of equal parts of 78% and 100% extraction wheat flour were used Milk was not added

In the preliminary trial (experiment I) three different specimens of bread were tested white wheaten bread fortified respectively with ferrum reductum to 40 mg iron per 100 g loaf and with the same amount of ferrous sulphate ($FeSO_4 \cdot 7H_2O$) and unenriched with 1 1 mg iron per 100 g The same sort of bread was used in experiment II with the exception of the unenriched kind. In experiment III both white wheaten and wholemeal bread were fortified with water free ferrous sulphate Chemical analysis showed a mean value of 3[illegible] mg of iron per 100 g white wheaten and 29 mg per 100 g wholemeal bread in duplicate measurements Analysis of naturally occurring phytates revealed a mean value of 9 mg of phytic acid phosphorus per 100 g white wheaten and 71 mg per 100 g wholemeal bread

Results

Preliminary trial

As shown in Table I there was a small but rather irregular increase in serum iron in most subjects after ferrous sulphate-enriched white wheaten bread In one man with posthemorrhagic anemia and in one healthy woman the increase was pronounced In four individuals however there was a small decrease

After ferrum reductum-enriched as well as unenriched white bread the increase in serum iron was minimal

In each individual there was a tendency though irregular to a better response after ferrous sulphate-enriched than after the ferrum reductum or unenriched white bread Most of the subjects in this experiment however were either healthy men or men with illness unrelated to iron deficiency In order to get a better evaluation of the absorption of iron young women with presumably a need for iron were used in the next two experiments

White bread enriched with ferrous sulphate vs ferrum reductum

Table II shows that in the ferrous sulphate experiment the increase in serum iron concentration was appreciable in all but one subject in whom there was a small increase only After ferrum reductum-enriched white bread there was also a marked increase in most cases although a decrease in two The mean increase in serum iron was 38 and 12 μg per 100 ml respectively In all but one subject the response was higher after ferrous sulphate than after the ferrum reductum sort

Ferrous sulphate-enriched white vs ferrous sulphate-enriched wholemeal bread

This experiment was conducted in order to assess the possible inhibitory action of dietary phytate on the iron absorption

As demonstrated in Table III there was an appreciable increase in serum iron after both kinds of bread The increase was however significantly higher after the white bread The mean

increase was 59 and 30 μg iron per 100 ml respectively

B UTILIZATION STUDIES

A controlled clinical trial with registration of changes in hematological indices was performed in a group of adult women who over a 12 weeks period were given bread fortified with ferrous sulphate in a moderate dose

Material and Methods

One hundred and forty six adult female patients in three units of a mental hospital in Oslo were divided into two groups which for practical reasons had to be based on the hospital units 103 patients with a mean age of 5..7 years in the experimental group and 43 patients with a mean age of 45 8 years in the control group

The patients in the selected experimental group were older were in a more serious psychiatric state had relatively low physical activity and calorie consumption and their initial Hb and hematocrit (Hct) levels were higher For these reasons they were unlikely to have a more beneficial effect from dietary iron supplement than the subjects in the control group

In the course of the trial 49 subjects dropped out 6 in the experimental group and .3 of the controls This was due to discharges transfer to other units and in some cases due to the administration of iron by the hospital s own staff Consequently the subsequent tables and discussion relate only to the patients who completed the trial 77 subjects in the experimental group and only 0 in the control group

The patients in the experimental group received white wheaten and rye bread fortified with ferrous sulphate for 12 weeks starting in January 1967 The controls were given the same types of bread but unenriched

The white bread was made of 78% extraction wheat flour with the addition of hand skimmed milk The recipe of the rye bread included 55 parts of 75% extraction rye flour 5 parts of 78 % extraction wheat flour 10 parts of 75% extraction wheat flour 5 parts of 100% extraction wheat flour and 5 parts of whole wheat grains Milk was not added Ferrous sulphate ($FeSO_4 7H_2O$) was added to ensure a total amount of approximately 10 mg iron per 100 g of both white and rye bread Chemical analysis of the fortified bread however revealed only approximately 8 mg per 100 g Analysis of the unfortified varieties showed 1 1 mg iron per 100 g rye bread and 0 8 mg per 100 g white bread The phytate content was 34 m of phytic acid phosphorus per 100 g iron fortified rye bread .0 mg per 100 g unfortified rye bread and ?2 mg per 100 g unfortified white bread All breads were baked by the same baker throughout the experiment at the hosp tal s own bakery The iron fortification procedure had no ad erse effects on the baking process as such

Before the start of the experiment and after 6 and 12 weeks, capillary blood was taken from the fingertip by a specially trained nurse who determined the Hb Hct and MCHC values by the methods previously reported (9)

Table IV *The mean values of hemoglobin hematocrit and MCHC before and during the experiment in the experimental group and the control group*

Time of examination	Experimental group (77 pat) Mean age 54 3 y Hb (g%)	Hct ( )	MCHC (%)	Control group (?0 pat.) Mean age 45 8 y Hb (g)	Hct (%)	MCHC ( )
Before the experiment	13 96	41 29	33 87	13 53	39 70	34 10
After 6 weeks	14 08	41 43	34 00	14 22	40 80	34 84
After 12 weeks	14 32	41 71	34 34	13 86	40 55	34 33

Results

Table IV shows a small but steady increase in the mean Hb concentration in the experimental group during the experiment The mean increase after 12 weeks was 0 36 g % which is statistically significant at the 5% level ($t = 2\ 305$ $0\ 01 < p < 0\ 05$) After the end of the trial the mean Hb value was 14 32 g% which is identical with the mean normal value for healthy Norwegian women (13)

The relative distribution of the Hb values in the experimental group before and after 12 weeks of iron fortified bread is demonstrated in Fig 1 Before the experiment five subjects or 6 5% had a Hb value of less than 12 5 g% which is considered to be the lower normal value in adult women (13) After the trial only two women were below this value

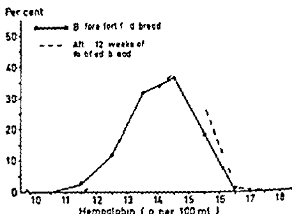


Fig 1 The relative d stribution of the hemoglobin va ues in the experimental group before and after 1 weeks of iron fortif ed whomeal and white bread

Table V *Difference in Hb concentration between values obtained after 12 weeks of iron fortified bread and values at the start of the trial in the experimental group*

Difference in Hb (12 weeks—before) g/100 ml	No of subjects		Per cent	
(+1 5)—(+2 4)	5	32	6 5	41 6
(+0 5)—(+1 4)	27		35 1	
(−0 5)—(+0 4)	40		51 9	
(−1 5)—(−0 6)	5		6 5	
Total	77		100 0	

Table V shows the individual increase or de crease in Hb con entration during the trial Nearly half of the subjects had an increase of more than 0 5 g° compared with five out of 77 with a corresponding decrease in Hb concentration

In the experimental group there was also a moderate increase in the mean Hct and MCHC values as shown in Table IV

Due to great losses of subjects in the control group a detailed analysis of the data has not been carried out As shown in Table IV there was an increase in the mean Hb Hct and MCHC values during the first six weeks of the trial This was followed however by a decrease in all indices during the last six weeks period The increase in the mean Hb value during the first six weeks was 0 69 g / which is statistically sig nificant ($t = 2\,958$ $p < 0\,01$) but the endpoint after 12 weeks was not significantly different from the initial value (increase = 0 33 g o $t =$ 1 167 $0\,2 < p < 0\,5$)

DISCUSSION

In this study postabsorption serum iron level has been used to estimate the relative absorption of different iron compounds using the test person as his own control Taking the increase in serum iron two hours after the ingestion of iron fortified white bread as an expression of the level of ab sorption it was demonstrated that ferrous sul phate gave a better response than ferrum re ductum (Tables I and II)

It was also found that the inhibitory effect of dietary phytate on the absorption of ferrous sul phate was only partial as a marked increase in serum iron was observed after enriched whole meal bread containing 71 mg of phytic acid phosphorus per 100 g although not to the same extent as after enriched white wheaten bread containing only 29 mg (Table III) The basing our conclusions on the increase in serum iron two hours after the ingestion of iron fortified white or wholemeal bread as an expression of absorption may be criticized as the rate of ab sorption may vary between the compounds used Because of this relative uncertainty in the evalua tion of the absorption experiments a controlled clinical trial with ferrous sulphate-enriched white and wholemeal bread and with registration of changes in hematological indices was considered inevitable

The results of the clinical trial (Table IV) show that the bread fortified with ferrous sul phate to 8 mg per 100 g produced a significant rise in the mean Hb value of the adult female patients up to the mean optimal level for Nor wegian women (13) There was a corresponding shift towards higher Hb values in the distribution curves (Fig 1) and nearly 50° of the subjects had an increase of more than 0 5 g° (Table V) There was also a concomitant increase in the Hct and MCHC valu s (Table IV) It appears therefore that the iron used for fortification was utilized to an appreciable extent

Unfortunately a great drop out of controls during the experiment reduced the size of this group to only 20 subjects For this reason the interpretation of these results is difficult After a significant rise in the mean Hb value during the first six weeks period there was a pronounced fall during the last six weeks Th total increase of the mean Hb value after 12 weeks was not statistically significant and the endpoint was only 13 86 g compared with the optimal level of 14 32 g° reached in the experimental group

Considering the absorption experiments and the clinical trial together however it appears that ferrous sulphate baked into wholem al and white bread can b absorbed and utilized for hemoglobin synthesis This is in good agreement with the favorable results reported by Stott in his long term feeding trial (18) Elwood however found it difficult to accept the conclusions of Stott without considerable reservations and he was furthermore of the opinion that all other previous long term studies suggest that iron baked

increase was 59 and 30 μg iron per 100 ml respectively

B UTILIZATION STUDIES

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Table IV *The mean values of hemoglobin hematocrit and MCHC before and during the experiment in the experimental group and the control group*

	Experimental group (77 pat) Mean age 54 3 y			Control group (20 pat.) Mean age 48 8 y		
Time of examination	Hb (g)	Hct ( )	MCHC (%)	Hb (g%)	Hct (%)	MCHC ( )
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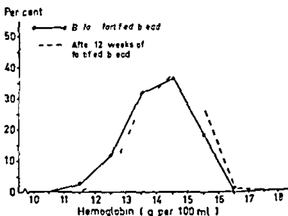


Fig 1 The relative distribution of the hemoglobin values in the experimental group before and after 12 weeks of iron fortified wholemeal and white bread

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THYROCALCITONIN

Method of Extraction and Effect on the Calcium Content of the Blood

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Abstract Thyrocalcitonin is a hypocalcaemic factor formed in the C cells which are mainly concentrated in the thyroid gland By inhibition of bone resorption thyrocalcitonin lowers the calcium concentration in the blood

The authors have isolated thyrocalcitonin from thyroid tissue of pigs and from 4 out of 8 human thyroids removed surgically because of goitre Two of these four goitres were diffuse and toxic and two were nodous and toxic

The technique of preparation of thyrocalcitonin is based on fractioning on columns of Sephadex G 50 and G 75 on a hydrochloric acid extract from thyroid tissue after salt precipitation of the inactive high molecular proteins whereby the preparation is purified from 50 to 100 times

With respect to magnitude and duration the fall in the serum calcium concentration is proportional to the dose of thyrocalcitonin administered Five hours after subcutaneous injection of from 1 to 2 units of thyrocalcitonin the serum calcium concentrations are higher than the zero values Most likely this indicates that the parathyroid glands surpass their compensating duty

The response to thyrocalcitonin in rabbits is different from that observed in rats the reduction in the serum calcium concentration being much more prolonged in the rabbit This is most likely caused by the fact that the parathyroid reserve of these animals is low

In 1962 Copp et al (8) found that a hormone having a hypocalcaemic action is secreted from the parathyroids in response to hypercalcaemia They named the new hormone calcitonin In 1963 Hirsch et al (16) discovered a hypocalcaemic hormone of the thyroid gland and called it thyrocalcitonin

Today most investigators consider these two hormones to be identical and it is generally agreed that the thyroid is the main contributor to the hypocalcaemic response to a hypercalcaemic stimulus Recently several authors independently have shown that the hormone is produced in the so-called C cells (7 22) These cells originate from the ultimobranchial body a structure in foetal life At an early stage of development the C cells in most animals fuse into the thyroid and the parathyroid which explains how the hormone can be extracted from both glands

Thyrocalcitonin activity has been demonstrated in extracts of thyroid tissue of the rat rabbit dog hog ox monkey calf and goat (10 17) but there is immunological evidence of a structural difference between thyrocalcitonin from different animal species (25) The presence of thyrocalcitonin in the human thyroid has been shown by several investigators (3 17 21) The hormone has been highly purified from pig thyroids and identified as a polypeptide with a molecular weight of about 4500 (5 15 23)

Mode of action The effect is not due either to an action on the parathyroid gland or to an inactivation of the parathyroid hormone since thyrocalcitonin is effective in parathyroidectomized rats (1 14 16) A significant thyrocalcitonin effect has been shown in rats after nephrectomy (14 17) and after removal of the gastrointestinal tract (1) which proves that these organs are not essential mediators of the hypocalcaemic effect An enhanced soft tissue uptake of calcium does not appear to be involved in thyrocalcitonin action (6 14) Many experiments show that thyrocalcitonin acts directly on bone by inhibiting resorption Milhaud et al (20) were the first to suggest this on the basis of their isotope studies on rats The hypothesis was supported by Aliapoulios et al (2) and by Friedman and Raiz (13) who showed independently and by different procedures that thyrocalcitonin diminishes the

resorption of bone in tissue cultures It was demonstrated most clearly when bone resorption was stimulated by parathyroid hormone Martin et al (19) found that urinary excretion of hydroxyprolin—a measure of collagen breakdown and thus of bone resorption—was reduced in rats receiving thyrocalcitonin Foster et al (12) examined the caudal vertebrae in parathyroidectomized rats receiving daily injections of thyrocalcitonin for one month and they found increased trabecular bone in the metaphyses

In addition to the calcium lowering effect thyrocalcitonin produces a fall in plasma phosphate concentration (14 17) This can be explained by the inhibition of bone resorption Robinson et al (24) have shown that in the kidney thyrocalcitonin acts synergistically to parathyroid hormone by increasing the phosphorus excretion

The serum concentration of magnesium seems to be unaffected by thyrocalcitonin (10 11 14) Milhaud et al (21) were the first to administer the hormone to human beings After an intravenous dose of purified pig thyrocalcitonin they found a slight but statistically significant fall in the serum calcium concentration of four persons Foster et al (11) induced a more distinct fall in three patients suffering from hypercalcaemia complicating disseminated malignant diseases

MATERIAL AND METHODS

Fresh pig thyroids were minced and defatted by repeated extraction with cold acetone All the procedures described in the following took place at a temperature of 4 C for the purpose of maintaining the biological activity The defatted thyroid tissue was minced in a Waring blender in 0 1 N hydrochloric acid (10 ml of hydrochloric acid g thyroid tissue) After one hour the mixture was centrifuged at 1 00 rpm for one hour The high-molecular proteins were then precipitated by adding 3 M sodium chloride to a final concentration of 0 75 M sodium chloride Twenty four hours later the solution was centrifuged at 1500 rpm for one hour The inactive precipitate was discarded Further purification was achieved by fractionation on columns with Sephadex G-50 and G-75 An 0 05 N acetate buffer pH 3 8 was used as elution agent flow rate approximately 25 ml h, height of column 85 cm and diameter 2 5 cm. The fractionation was carried out with an LKB collector connected to a Uvicord absorptiometer the absorption at 280 mμ being recorded with an LKB recorder

The experimental animals used were rats of the type Ss Vs.Hs from the State Serum Institute weight 180-200 g, and rabbits of the usual Danish Country Stock

The extracts were injected subcutaneously Blood samples were obtained by heart puncture under aether anaesthesia immediately before and between one and five hours after the injections of extract

In order to assess the potency the authors established that one unit was the volume which when injected subcutaneously into a rat weighing 180 g, would produce a fall of 1 mg/100 ml in the serum calcium concentration one hour after injection Hence this unit is of the same order of magnitude as that defined by Hirsch et al (17) although we do not as did these investigators, use a standard preparation

The calcium concentrations were determined by photometric titration with EDTA as described by Fales The coefficient of variation is 1 / *

Protein determinations were made by the Kjeldahl method

RESULTS

After subcutaneous injections into 40 rats of 1.5 ml of crude extract from four batches prepared consecutively a reduction in the serum calcium concentration of 1 60 mg/100 ml ± 0 55 mg ($p < 0\ 001$) was observed after one hour The average content of protein in the crude extract was 370 mg of protein nitrogen per 100 ml After injection into eight rats of a similar volume of the extraction solution (0 1 *N* hydrochloric acid) a slight increase in the serum calcium concentration of 0 18 mg ± 0 14 mg ($p < 0\ 01$) was seen

Fig 1 shows that the extension and duration of the reduction in the serum calcium concentration is proportional to the size of dose injected

In Fig 2 the individual reductions observed in the rats after injection of 1 6 units of thyrocalcitonin are shown and it appears that rather large individual variations occur in response to thyrocalcitonin The rats were of the same sex (male) and weight (180 g) and on the same diet Intravenous injection was tried instead of the subcutaneous method and the same conditions were found

Rabbits behave differently from rats after administration of thyrocalcitonin the reduction in the serum calcium concentration occurring at a slower rate and being maintained for a longer period of time as appears from Fig 3

Following salt precipitation with sodium chloride as described in the foregoing the concentration of thyrocalcitonin per mg of protein is increased 2 to 5 times By way of example it should be mentioned that in six rats each of which received an injection of crude extract containing 3 30 mg of protein nitrogen an average

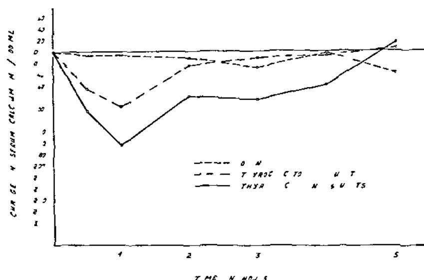


Fig 1 Serum calcium in rats (wt 180 g) after injection of 0 1 N HCl and thyrocalciton n in a dose per animal of 1 unit and 1 6 *units respectively Each rat* received the same volume (1.5 ml)

reduction in s rum calcium con^entration of 2 12 mg/100 ml $\pm$ 0 39 mg was observed or a reduction of 1 00 mg/100 ml p r 1 56 mg of protein nitrogen After salt precipitation injections of 0 51 mg of protein nitrogen produced an average reduction in six rats of 1 26 mg of calcium/100 ml $\pm$ 0 14 mg or a reduction of 1 00 mg/100 ml per 0 40 mg of protein nitrogen—an almost four fold increase in activity

Filtration on Sephadex G 50 of the supernatant after salt precipitation produces an extinction curve of the type shown in Fig 4 The thyrocalcitonin is to be found in the descending part of the 2nd peak The 1st peak consists of high molecular proteins the 2nd peak of low molecular proteins and the 3rd peak of nucleotides and salts

By this method of fractionation a thyrocalcitonin preparation is obtained which is from 50 to 100 times purer than the crude extract Injection of 0 03 mg of protein nitrogen from the most potent Sephadex fractions of an extract when injection into six rats of 2 80 mg of crude extract protein nitrogen produced a reduction in the

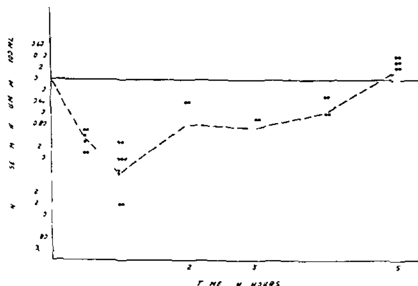


Fig The ind vidual response in rats to a subcutaneous injection of the same amount of thyrocal c tonin (1 6 units)

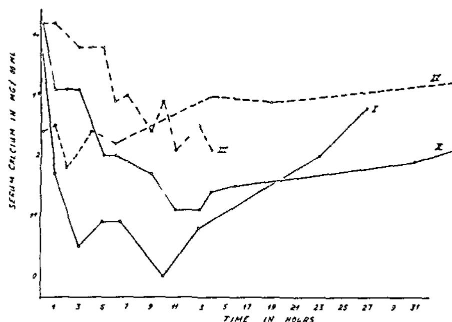


Fig 3 The effect on serum calcium of a subcutaneous injection of thyrocalcitonin in three non fasting rabbits. Rabbits nos I and II were given thyrocalcitonin of porcine origin (1 unit/100 g body weight) while rabbit no III received a similar dose of human thyrocalcitonin and rabbit no IV an equal volume of buffer solution

serum calcium concentration of 1 30 mg/100 ml ±0 04 mg was capable of lowering the serum calcium concentration in five rats by 1 22 mg/100 ml—an increase in activity of almost 90 times

The same technique was employed in the examination of eight human thyroid glands removed surgically because of goitre They represented six cases of diffuse toxic goitre and two cases of nodous toxic goitre Hypocalcaemic activity was found in crude extracts from both of the latter cases and from two of the former

One of the crude extracts from a diffuse toxic goitre was examined more closely The crude extract contained 144 mg protein nitrogen/100 ml Subcutaneous injection of 1 5 ml into four rats produced a reduction in the serum calcium concentration of 0 85 mg/100 ml ± 0 20 mg Following salt precipitation 40 ml of the supernatant was fractioned on a column of Sephadex G 75 in the same way as the pig thyroid extract described in the foregoing The extinction curve resembled that shown in Fig 4 the hypocalcaemic activity being distributed into three fractions in the descending part of the 2nd peak In these fractions protein nitrogen concentrations of 20 11 and 3 mg respectively were found Injection of 1 ml of these fractions into six rats produced an

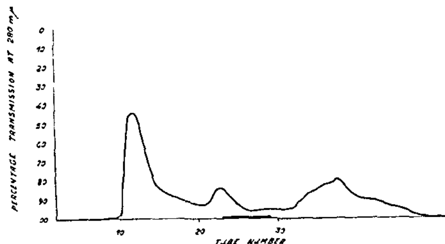


Fig 4 Gel filtration on Sephadex G 50 of a pig thyroid extract after NaCl fractionation 0 05 N acetic acid was used as eluant Flow rate 4 ml/h 1? ml fractions were collected The activity was found in tubes 3–5

average reduction of 0 28 mg/100 ml$\pm$0 03 mg The three fractions concerned were pooled (the protein concentration was determined at 8 mg protein nitrogen per 100 ml) and 25 ml were injected subcutaneously into a rabbit weighing 3000 g A protracted reduction in the serum cal cium concentration was seen maximum about 2 mg/100 ml as shown in Fig 3 Unfortunately the serum calcium values were not followed for a sufficiently long period of time

DISCUSSION

It was possible to confirm that the thyroid gland contains a hypocalcaemic factor—thyrocalcitonin The authors have examined thyroid tissue only from pigs and human beings but other investi gators have demonstrated the presence of thyro calcitonin in a great many experimental animals

The methodology of preparation is explained Fresh defatted thyroids were minced in hydro chloric acid After centrifugation the crude extract was purified by salt precipitation with sodium chloride and fractionation on Sephadex columns whereby a preparation was obtained having a purity 50 to 100 times higher

After subcutaneous injection of thyrocalcitonin into rats a maximum reduction in the serum cal cium concentration occurred after about one hour The magnitude and duration of the reduction is proportionate to the dose administered Like sev eral other investigators we found a pronounced individual sensitivity from one rat to another (3) Various working groups have each defined their own thyrocalcitonin unit Both Kumar et al (18) and Hirsch et al (17) used standard preparations characterized by their content of protein as com pared with their hypocalcaemic activity The un known thyrocalcitonin extracts were injected into experimental rats and the reduction in the serum calcium concentrations was compared with a curve illustrating the serum calcium concentra tion after injection of decreasing volumes of the standard preparation Hirsch et al (17) were the first to define a unit and in order to approach this unit as closely as possible we decided to establish a unit as the volum which produced a reduction in the serum calcium concentration of 1 mg/100 ml one hour after subcutaneous in jection into a rat weighing 180 g

The position of thyrocalcitonin in the extinc tion curve appearing by filtration on S phadex columns confirms the fact that it is a low molec ular protein Several investigators have found a molecular weight of about 4500 Thyrocalcitonin is rapidly decomposed by pepsin and trypsin it is fairly thermostable at pH 4 6 but is rapidly destroyed on being boiled in a strong acid or alkaline environment (4)

Aliapoulios and Munson (1) showed that the hormonal effect is maintained for a longer period of time in parathyroidectomized rats in which the reverse effect of the parathyroid hormone cannot exert any influence Similar conditions are found in intact rabbits the normal serum calcium con centrations of which strangely enough range around 15 mg/100 ml The reserve of the para thyroid gland is presumably low in these animals From Fig 1 it appears that five hours after injec tion of thyrocalcitonin into rats the serum cal cium concentration is higher than the zero value supposedly an indication of the fact that the para thyroid glands surpass their compensating duty

By exploring the thyrocalcitonin content in human thyroid tissue removed surgically we found this substance in two out of six cases of diffuse toxic goitre and in both cases of nodous toxic goitre examined by us The purification and the hypocalcaemic effect in the rat and the rab bit of one of the human thyrocalcitonin extracts are discussed in further detail

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PULMONARY FUNCTION IN FORMER ENDURANCE ATHLETES

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Abst act Pulmonary function was studied in 57 former champion endurance runners or cross-country skiers A control group of 53 non athletes of the same age was submitted to a similar study Although the results of various pulmonary function tests in the total groups were also compared the main emphasis was laid upon the comparison of pulmonary function in 28 healthy controls and 37 healthy athletes who were free from detectable cardiovascular respiratory or other s gnificant d sease

The former athletes differed from the control subjects by having a larger vital capacity (VC) and total lung capacity (TLC) Res dual volume (RV) and functional residual capacity (FRC) as well as RV/TLC × 100 and FRC/TLC × 100 were slightly but significantly greater in athletes than in controls

Maximum voluntary ventilation (MVV) tended to be greater in athletes than in control subjects Forced ex piratory volume in 1 5 sec as a percentage of VC (FEV) was approximately similar in controls and athletes

Pulmonary d ffusing capacity (D co) at rest was markedly greater in athletes than in control subjects

MVV showed a significant negative correlation to age in healthy controls and athletes Although the values for MVV tended to be higher in athletes than in controls the difference in the level of the regression lines to age was not sign f cant and the slopes of the regression lines in the two groups did not differ significantly FEV was negatively correlated to age in healthy athletes but not in control subjects A signif cant negative correla tion was also observed between D co and age in healthy athletes but not in controls

D co showed a s gnificant positive correlation to VC in healthy control subjects and in healthy athletes the correlation between these two parameters almost reached statistical significance In athletes a s gnificant positi e correlation was observed between D co at rest and maximum oxygen uptake pred cted on the basis of heart rate response to a submaximal work load In control subjects the correlation between $D_{L,CO}$ and maximum oxygen uptake was not statistically sign f cant MVV also showed a s gnificant positive correlation to maximum oxygen uptake in athletes but not in control subjects

Pulmonary function in actively competing athletes has been studied extensively However the re sults have not been entirely uniform The vital capacity in athletes has been reported to be larger than in non athletes by some investigators (31 36 38) whereas in most studies no consistent increase has been observed (2 15 27 29 35) Champion swimmers seem to form a special group of athletes having definitely large vital ca pacities (3 28 31 34 35) The total lung capac ity of athletes has been the subject of fewer studies no consistent enlargement has been ob served (27 29)

Ventilatory dynamics being influenced by muscular factors (5) have in most studies be n found to show differences between athletes and non athletes Thus the maximum voluntary ven tilation has been found to be greater in well trained athletes than in non trained subjects (15 34 35) The forced expiratory volume of athletes has been reported to be either increased (15 28) or comparable to that observed in non athletes (27)

The diffusing capacity of the lungs ($D_{L\,CO}$) measured at rest (21) or during exercise (23) has been shown to be correlated to aerobic working capacity in studies of subjects representing a wide range of degrees of physical fitness The pul monary diffusing capacity at rest has been re ported to be increased in one study of well trained athletes (7) while in another no signifi cant increase was found (29) On the other hand the diffusing capacity during exercise has been observed to be consistently increased in well trained athletes (7 25 29)

With advancing age the pulmonary function tends to deteriorate Vital capacity and total lung capacity decrease residual volume and functional residual capacity both the absolute values and the values expressed as percentage of total lung capacity tend to increase maximum voluntary

ventilation decreases and the rate of air flow during forced expiration decreases (6 8 10 16 20) The diffusing capacity of the lungs also decreases with age (12 18 24 30 32)

Endurance athletes especially long-distance runners and skiers have during their active years subjected themselves to intense training and they often maintain a high level of physical activity even after stopping active competition A study of pulmonary function in middle aged and older endurance athletes is of interest in order to observe whether the age-dependent deterioration of pulmonary function differs in former endurance athletes and in men of comparable age who have lived a more sedentary life In the following we report the results of pulmonary function studies in 57 former endurance athletes 27 long-distance runners and 30 cross-country skiers These men all had belonged to the élite of Finnish athletes and had also distinguished themselves in international competitions They are compared with a group of 53 non athletes of similar age

MATERIAL AND METHODS

The present study is part of a more extensive project the principal aim of which was to make an evaluation of cardiovascular and pulmonary function in former endurance athletes as compared with that in non athletes of the same age The invitation to take part in the study was sent to 93 former champion athletes aged 40 or more The final group of athletes studied comprised 61 men Complete pulmonary function tests were obtained on 57 athletes 7 runners and 30 skiers For practical reasons a group of men living in Helsinki was selected as a control group An attempt was made to choose them from a social group not too far from that of the majority of athletes, and so that the age distribution of the control group would correspond to that of the athletes Forty four of the 54 control subjects were members of the Association of Shopkeepers in Helsinki and participated on a voluntary basis To improve age matching ten further control subjects were selected among men attending an annual executive health check up at the Institute of O cupational Health In the selection of control subjects men whose occupation required a high degree of physical activity were excluded Pulmonary function studies were available on 53 control subjects

The examination procedure and the results concerning the occurrence of cardiovascular and other significant diseases in the athletes and controls the various cardiovascular characteristics in the two groups as well as the results of anthropometri measurements have previously been described (33) The previous paper also includes a detailed analysis of the current physical activity and smoking habits of the athletes and controls particularly in relation to cardiovascular disease and age

The lung volumes and the maximum voluntary ventilation were recorded with a Collins spirometer Functional residual capacity and residual volume were determined by the closed circuit helium-d lution technique The determination of lung volumes was carried out in the supine position and the MVV_F was measured in the upright position Spirometric volumes were corrected to BTPS In addition to absolute values vital capa ity total lung capacity and maximum voluntary ventilation were expressed as percentages of predicted normal values (6) Forced expiratory volume in 1.5 sec ($FEV_{1.5}$) was used in the analysis of the forced expirogram instead of the FEV commonly used The bell of the spirometer used was rather heavy and the resistance of the tube system was not optimally low for the recording of the expirogram With our equipment the FEV , approximately corresponds to the FEV recorded with spirometers possessing more favourable characteristi s for the recording of the expirogram

The pulmonary diffusing capacity of carbon monoxide ($D_{L\,CO}$) was measured in the sitting posture by using the modified Krogh breath holding technique described by Forster et al (13) and Ogilvie et al (30) The gaseous mixture used in measuring $D_{L\,CO}$ contained 0 259% of carbon monoxide and 9 ?7% helium in air The dilution of helium was measured with a catharometer (Cambridge Instrument Co) and the carbon monoxide analyses were performed with Infra Red Gas Analyser (Sir Howard Grubb Parsons & Co Ltd Newcastle upon Tyne) The results are expressed as absolute values ml/min × mm Hg and as percentages of predicted normal values, which eliminates variation due to age and lung size The normal values were derived from the equation

$$D_{L\,CO} = 24\,40 - 0\,179\,x_1 + 3\,510\,x_2$$

where x_1 = age (years) and x = vital capacity (litres) This equation is based on the results in 1 6 healthy Finnish men (18) The reliability of the $D_{L\,CO}$ determination by this technique has been analysed in a previous paper from this laboratory (19)

The maximum oxygen uptake (aerobic capacity) was estimated by means of the nomogram of Åstrand and Ryhming (4) from the heart rate response to standard exercise on a bicycle ergometer The subject pedalled at a work load of 900 kg m min for 4 min and the heart rate during the last 30 sec of the exercise was used for the estimation of the maximum oxygen uptake Correction factors for age given by Åstrand (1) were employed in the calculations A detailed report on the exercise response of healthy control subjects and athletes has been given in the previous paper (33)

The criteria proposed by Higgins ( ) were used in the* diagnosis of chronic bronchitis

RESULTS

General Characteristics of the Control Subjects and Athletes

General characteristics of the present series have been described in detail in the previous paper

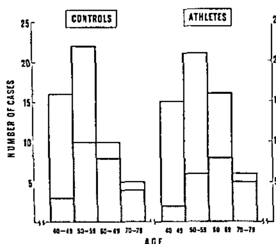


Fig 1 Distribution of the series according to age White part of each column indicates the number of individuals considered to be free from cardiovascular respiratory or other significant disease

(33) Therefore only the data pertinent to the results of the pulmonary function tests will be summarized here

Age

The age distribution of the control subjects and athletes is shown in Fig 1 The mean age of the control subjects was 55 2 with a range of 41 to 78 years and that of athletes 55 8 with a range of 40 to 79 years The mean age of runners was 59 6 with a range of 43 to 79 years while the mean age of skiers was lower 52 3 with a range of 40 to 72 years

Occupation

None of the control subjects belonged to occupational classes associated with a high degree of physical activity whereas in the group of athletes 22 of the 57 athletes had an occupation requiring a high level of physical activity

Diagnostic classification

Twenty eight of the 53 control subjects (53°) and 37 of the 57 athletes (65) were considered to be healthy i e free from detectable cardiovascular respiratory or other significant disease Probably the criteria employed in this study in the definition of healthy subjects were more strict than those employed in the selection of many of the "normal materials upon which the predicted normal values of pulmonary function tests are based

The diagnostic classification of the remaining control subjects and athletes has been pres nted in the previous paper Coronary heart disease and hypertension were the most common diagnoses Congestive heart failure of mild or moderate degree was diagnosed in one control subject and in three athletes The diagnosis of chronic bronchitis of mild degree was made in six control subjects and in two athletes

Table I *Smoking habits*

	Whole series		Healthy subjects	
	Controls	Athletes	Controls	Athletes
Non smokers	13	25	6	11
Ex smokers	20	16	10	10
<20 cigarettes/day	11	9	8	9
>20 cigarettes/day	9	7	4	7
Total	53	57	28	37

Physical activity

The current physical activities of control subjects and athletes were arbitrarily classified into four grades Irrespective of the presence or absence of disease the average d gree of physical activity was greater in athletes than in controls Twenty one of the 53 control subjects (40) were classified at activity levels III or IV while in the group of athletes 39 of the 57 men (68) belonged to these grades

Smoking habits

Classification of the whole series and healthy subgroups according to smoking habits is shown in Table I In the whole series there were fewer non-smokers in the control group than in the group of athletes However in the healthy subgroups this difference tended to level off

Anthropometric measurements

In the whole s ries the mean weight height and subscapular skinfold thickness were slightly but significantly greater in controls than in athletes The healthy subgroups of controls and athletes did not differ in regard to height and weight but the mean skinfold thickness was significantly

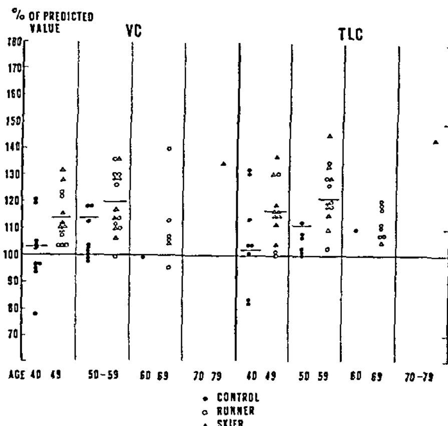


Fig Individual values for vital capacity (VC) and total lung capacity (TLC) as percentages of predicted values in healthy control subjects and athletes in various age groups Horizontal lines indicate mean values ● control O runner Δ skier

greater in controls than in athletes These differences in body measurements between the groups were however so small that they obviously did not influence the results of pulmonary function tests

The Results of Pulmonary Function Tests

The results of various pulmonary function tests are given in Table II Although the main emphasis was laid upon the comparison of the healthy control subjects and athletes the total groups were also compared Both in the total group of control subjects and in the total group of athletes there were subjects who had illnesses which are known to affect respiratory function In spite of this the total groups reveal the same trends as those observed in the healthy subgroups

Lung volume and its subdivisions

As shown in Table II the *total lung capacity* (TLC) both as absolute value and as percentage of predicted value was significantly larger in athletes than in controls This applies to the total group as well as to the healthy subgroup Fig 2 shows the individual values for TLC as percentages of predicted values in healthy control subjects and athletes in various age groups. In the 40–49 and 50–59 age groups the number of subjects was sufficiently great to allow statistical analysis of the results within the age groups In the 40 to 49 age group TLC was significantly greater in athletes than in controls ($p < 0.0?$) but in the 50–59 age group the difference was not significant

In the whole series the *vital capacity* (VC) was similarly larger in the athletes but in the healthy subgroup only the difference in the values expressed as percentage of predicted values reached significance

Fig 2 shows the individual VC values as percentages of predicted values in healthy controls and athletes in various age groups In the 40–49 age group VC was significantly greater in athletes than in controls (p 0.02) whereas in the 50–59 age group the difference between the groups was not significant

Residual volume (RV) and *functional residual capacity* (FRC) expressed as absolute values

Table II *Results of pulmonary function tests*

	Whole series					Healthy subjects				
	Controls (n 53)		Athletes (n = 57)			Controls (n = 28)		Athletes (n = 37)		
	Mean	S D	Mean	S D	p	Mean	S D	Mean	S D	p
Vital capacity (VC) l	3 84	0 7	4 31	0 6?	<0 001	4 12	0 61	4 35	0 49	—
VC of predicted	101 2	16 1	117 0	14 4	<0 001	108 3	14 3	116 7	11 7	<0 05
Residual volume (RV) l	1 54	0 51	1 86	0 38	<0 001	1 54	0 49	1 85	0 36	<0 01
Functional residual capacity (FRC) l	2 11	0 64	2 53	0 55	<0 001	2 16	0 72	2 56	0 52	<0 0?
Total lung capacity (TLC) l	5 38	1 03	6 12	0 82	<0 001	5 67	1 02	6 0	0 68	<0 05
TLC of predicted	101 5	17 0	117 8	14	<0 001	106 5	17 0	118 6	11 7	<0 01
RV/TLC 100	?8 3	6 4	30 5	4 8	<0 05	?6 7	4 8	9 8	4 2	<0 01
FRC/TLC 100	38 8	7 5	41 2	6 0	—	3 2	7 1	41 2	5 9	<0 02
Maximum voluntary ventilation (MVV_F) l/min	121 3	30 8	133 8	31 7	<0 05	1?9 9	31 7	139 0	30 6	—
MVV_F of predicted	109 9	24 0	125 3	4 4	<0 01	114 2	26 2	126 ?	24 2	—
Forced expiratory volume in 1 5 sec % of VC (FEV)	75 5	8 3	77 1	8 5	—	?5 7	8 8	77 8	7 5	—
Diffusing capacity ($D_{L\,CO}$) ml/min mm Hg	28 3	5 4	33 9	5 7	<0 001	29 5	4 9	34 7	4 8	<0 001
$D_{L\,CO}$ of predicted	101 4	16 7	115 9	16 9	<0 001	99 4	14 2	115 0	13 7	<0 001

were significantly greater in athletes than in controls both in the whole series and in the healthy subgroup

RV/TLC × 100 was slightly but significantly greater in athletes than in controls both in the whole series and in the subgroup of healthy subjects *FRC/TLC × 100* was significantly greater in athletes than in controls in the healthy subgroup only

Within the group of athletes there were no significant differences in the subdivisions of the total lung volume between runners and skiers

Ventilatory dynamics

Maximum voluntary ventilation (MVV_F) both as absolute value and as percentage of predicted value was significantly greater in athletes than in controls in the whole series In the healthy subgroup the differences did not attain significance Fig 3 shows the individual values for MVV_F as percentages of predicted values in healthy control subjects and athletes in various age groups In the 40–49 age group the mean values did not differ significantly but in the 50–59 age group MVV_F was significantly greater in athletes than in controls ($p < 0\ 02$)

Forced expiratory volume in 1 5 sec expressed as percentage of VC (FEV%) did not show any significant difference between controls and athletes

Within the group of athletes there was no significant difference in MVV_F or FEV% between runners and skiers

Pulmonary diffusing capacity

The mean values for $D_{L\,CO}$ both as absolute values and as percentage of the predicted value were significantly greater in athletes than in controls in the whole series and in the healthy subgroup Fig 3 shows the individual values for $D_{L\,CO}$ in healthy control subjects and athletes in various age groups Both in the 40–49 and 50–59 age groups the mean $D_{L\,CO}$ was significantly greater in athletes than in controls ($p < 0\ 01$) The highest $D_{L\,CO}$ values were observed in skiers aged 40–49 There were however only four runners in this group and therefore the values for runners and skiers could not be compared statistically In the 50–59 age group no definite difference in $D_{L\,CO}$ values was observed between runners and skiers

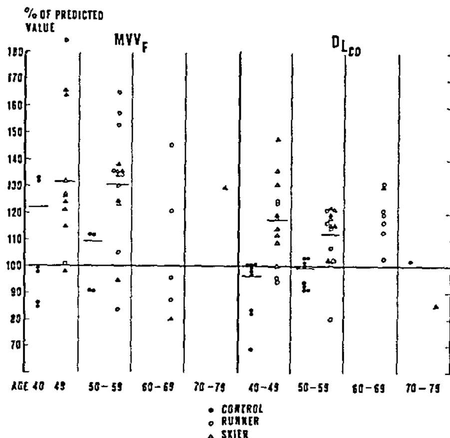


Fig 3 Individual values for maximum voluntary ventilation (MVV_F) and pulmonary diffusing capacity ($D_{L,CO}$) as percentages of predicted values in healthy control subjects and athletes in various age groups. Horizontal lines indicate mean values ● control ○ runner △ skier

Correlations Between Various Parameters in Healthy Control Subjects and Athletes

There was no statistically significant relationship between VC or other subdivisions of the lung volume and age either in the control group or in the group of athletes. MVV_F showed a significant negative correlation to age both in controls and athletes (Fig 4). Although the MVV_F values of athletes tended to be higher, neither the levels nor the slopes of the regression lines differed significantly. $FEV_{\%}$ showed a significant negative correlation to age in athletes ($r = 0.59$, $p < 0.01$) but not in control subjects. In athletes a significant negative correlation was observed between $D_{L,CO}$ and age (Fig 5). In the control group the correlation between these two parameters was not significant. $D_{L,CO}$ showed a significant positive correlation to VC in control subjects, and in athletes the correlation between $D_{L,CO}$ and VC almost reached the level of significance (Fig 6).

The aerobic capacity, as measured from the heart rate response to a standard work load (900 kg m for 4 min), was greater in athletes than in controls. In 24 control subjects who completed the test the mean *heart rate* at this work load was 139 beats/min (S.D. 15). The corresponding value for 36 athletes, 129 beats/min (S.D. 12), was significantly lower ($p < 0.01$). The mean *maximum oxygen uptake* estimated on the basis of these heart rate values was 2.80 l/min (S.D. 0.54) in controls and 3.12 l/min (S.D. 0.58) in athletes. The relationship between VC and maximum oxygen uptake almost reached the level of significance in athletes ($r = 0.32$, $0.10 > p > 0.05$), but in control subjects there was no relationship between these two parameters. TLC, RV and FRC showed no correlation to maximum oxygen uptake either in controls or athletes. MVV_F showed a significant positive correlation to maximum oxygen uptake in athletes ($r = 0.50$, $p < 0.01$) but not in control subjects. $FEV_{\%}$ showed no correlation to maximum oxygen uptake either in controls or in athletes. There was a significant positive correlation between $D_{L,CO}$ measured at rest and maximum oxygen uptake in athletes but not in control subjects (Fig 7).

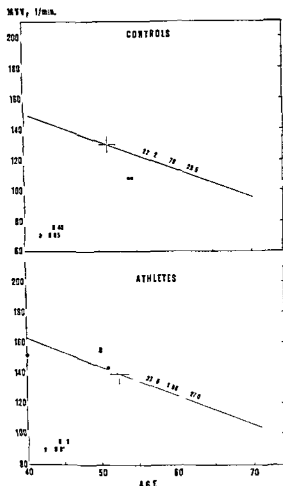


Fig. 4. The relationship between maximum voluntary ventilation (MVV_F) and age in healthy control subjects and athletes.

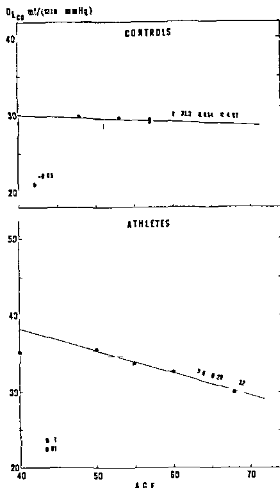


Fig. 5. The relationship between pulmonary diffusing capacity ($D_{L\,CO}$) and age in healthy control subjects and athletes.

DISCUSSION

The present series of former champion class endurance athletes is a result of multiple selection and may therefore not ideally represent the average of middle aged and older endurance athletes. The control group of the present study is even more selected, being merely a group of volunteers of suitable social class and age distribution and belonging to occupational classes which do not require a high level of physical activity. However, as stated when dealing with cardiovascular studies of the present material (33), it is impossible to define a control group corresponding to the group of former endurance athletes except in one respect, namely that the controls would not have a history of training for endurance athletics.

The group represented by champion athletes is formed at a young age. It is not known what are the somatic and psychological factors influencing the selection of champion athletes. In any case former champion class endurance athletes form a group which has once reached the upper limit of human physical endurance. The majority of the former athletes included in the present series had continued running or skiing as a hobby even after ceasing from active competition, and some of them in their fifties still took part in veteran competitions. The average level of current habitual physical activity of the group of athletes was therefore distinctly higher than that of the control group.

An important question is whether the control

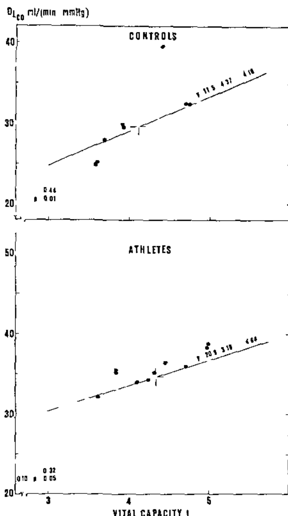


Fig 6 The relationship between pulmonary diffusing capacity ($D_{L\,C}$) and vital capacity in healthy control subjects and athletes

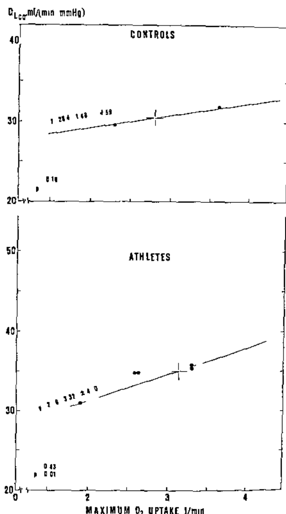


Fig 7 The relationship between pulmonary diffusing capacity ($D_{L\,C}$) and maximum oxygen uptake predicted from heart rate values at submaximal work load in healthy controls and athletes

group of the present study represents the average normal material of middle aged and older men in regard to pulmonary function The mean values of the results of various pulmonary function tests expressed as percentages of predicted normal values indicate that the control group evidently fulfils this criterion although the group is rather small especially when divided into subgroups according to age The mean values for the pulmonary function tests in the healthy subgroup of control subjects generally even exceeded the predicted normal values probably due to the strict criteria employed in the selection of healthy subjects Evidently the differences in pulmonary function observed between the former endurance athletes and control subjects are not due to a deviation of the present control series from th average of men of this age group The differences in smoking habits between control subjects and athletes were not great enough to explain the differences in the results of pulmonary function tests between the two groups

VC and TLC were found to be larger in former endurance athletes than in controls These findings are not in agreement with the results of several of the previous studies on young active endurance athletes in whom an enlargement of VC and TLC has not been consistently found One possible explanation of the result of the present study is that the decrease of VC and TLC

with a_e o~curs more slowly in physically active form r champion athletes than in non athletic m n of comparable age It may b~ significant that the greatest difference between athletes and controls was observed in the 40–49 age group (Fig 2) In a group of middle ag d and old r cross country runners who were well trained and still took part in veteran competitions Grimby and Saltin (15) found VC values above the predicted 100 per cent value in 10 of the 14 men aged 42–49 whereas in the 50–59 age group the VC values were below the predicted 100 per cent value in 10 of the 14 men

The finding of a larger RV and FRC in athletes than in controls is peculiar It obviously cannot be interpreted as an evidence of pulmonary emphysema in the clinical meaning of the word because MVV_F tended to be greater than normal in athletes and FEV^{0} was normal The large quantity of gases that constitute the FRC make it impossible for alveolar gaseous concentration to show wide variations in connection with changes in the rhythm of respiration It has been pointed out that the absence of this damping effect would affect the nervous control of respiration and readily result in periodic breathing of the Cheyn Stokes typ~ (17) Endurance athletes are known to breathe at a slower rate during exercise and often also at rest than untrained subjects With a slower and deeper rhythm of breathing a larger damping effect and consequently a larger FRC might in fact be needed

$D_{L\,CO}$ at rest was markedly greater in form r athletes than in controls This is in accordance with the difference observed in the predicted maximum oxygen uptake betwe n healthy athletes and controls (cf 21) In the healthy subgroup the difference in $D_{L\,CO}$ even reached a higher degree of significance than that in any of the other lung functions tested

Habitual physical activity appears to affect MVV_F more than VC Thus Yusa (39) observed in Japanese men doing heavy work (miners and stone-cutters) markedly increased MVV_F values but normal values for VC Heinonen et al (20) studied a group of Finnish firemen who are expected to maintain a high level of physical fitness independently of age The mean MVV_F of the firemen was found to exceed that of the Finnish control series by as much as 41 and the mean VC of firemen was 10 higher than in the control series On the other hand in a large group of non athletic men with varying physical activity Grimby and Soderholm (16) did not find any correlation between aerobic capacity as estimated from the heart rate at submaximal work load and VC MVV_F or $FEV_{1.0}$

In the present study no correlation was found in healthy control subjects between maximum oxygen uptake (aerobic capacity) predicted from the heart rate at a standard work load and VC MVV_F or $FEV_{1.0}$ However in healthy athlete MVV_F showed a significant positive correlation to maximum oxygen uptake and the correlation between VC and maximum oxygen uptak~ almost reached the level of statistical significance In healthy athletes a significant positive correlation was obs~rved between $D_{L\,CO}$ at rest and maximum oxygen uptake whereas in control subjects no significant correlation was observed between these parameters

The conclusion appears justified that an effect of habitual physical activity on the measured lung functions becomes manifest only in series which include trained subjects Among untrain d subjects an eventual association between habitual physical activity and lung function is covered by other randomizing factors

The volume of the functioning pulmonary capillary bed is an important determinant of pulmonary diffusing capacity It obviously depends on lung size and on the other hand on factors which affect the pulmonary circulation Both in the former athletes and in their controls a dependence of $D_{L\,CO}$ on VC was observable although more clearly in the controls On the other hand the association of $D_{L\,CO}$ and circulatory fitness evaluated by means of maximum oxygen uptake was closer in the former athletes The observations reported by Bjure (11) suggest that stroke volume may be a good approximation of the functioning pulmonary capillary bed becaus~ of th~ pulsatile character of the pulmonary capillary blood flow Although som what controversial data have been reported on the resting cardiac output in well trained athletes (9 14 26 37) according to most investigators the athletes have a large stroke volume at rest (9 14 37) and the most important characteristic of the central circulation in athletes is that stroke volume increases for a given work load more than in non athletes (9 14 26 37) Although studies of stroke

volume or other parameters of the central circulation were not made in the present investigation heart rate response to exercise indicated that *former champion athletes had a better circulatory* fitness than controls It is possible that former endurance athletes maintain at least partly the characteristic stroke volume of well trained athletes This could also explain the correlation between $D_{L\ CO}$ and maximum oxygen uptake predicted from the heart rate response to exercise in former athletes

VC and other subdivisions of the lung volume *which are known to change with age showed in* the present study no significant correlation to age either in healthy control subjects or in athletes This is obviously due to the small number of individuals especially in the older age groups However MVV_F showed a significant negative correlation to age both in healthy controls and athletes and FEV% was negatively correlated to age in athletes but not in controls $D_{L\ CO}$ similarly showed a negative correlation to age in healthy athletes but not in control subjects Podlesch and Stevanovic (32) have shown that in non athletic men the greatest decrease in $D_{L\ CO}$ occurs between 40 and 50 years whereafter the mean values remain essentially unchanged up to the age of 60–65 The results of the present study may indicate that in former endurance athletes $D_{L\ CO}$ does not decrease to the average $D_{L\ CO}$ level of middle aged non athletes until after the age of 60 As stated above this phenomenon might be explained by the fact that former endurance athletes usually maintain a good circulatory fitness up to a higher age than non athletic men

The present study is a cross sectional survey of the pulmonary function in middle aged and older endurance athletes Therefore it is not possible to decide on the basis of the results of this study whether the better results of pulmonary function tests in athletes than in non athletic men of the same age result entirely from a high degree of *previous* and current physical activity It must also be taken into account that some constitutional characteristics determining the efficiency of pulmonary function may be favourable for endurance training and thus influence the primary selection of future champion-class endurance athletes Follow up studies of pulmonary function in endurance athletes are needed In order to differentiate between the effect of factors influencing primary selection and the effect of vigorous endurance training and competition and *of a high level of physical activity* after stopping competition follow up studies should be started from the very beginning of the career of endurance athletes and then continued through the period of competition until old age Nowadays athletes are subjected to regular physiological studies With time therefore useful data for follow up studies of pulmonary function in athletes will accumulate in various centers

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CYSTIC DISEASE OF THE RENAL MEDULLA AND ITS POSSIBLE RELATION TO JUVENILE NEPHRONOPHTHISIS

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Abstract A 51 year-old man is reported with cystic disease of the renal medulla ushered in by polyuria and weakness followed by progressive uraemia with loss of salt and severe anaemia The urine contained no protein and was sterile and the sediment was normal The specific gravity of the urine was less than 1 010 Necropsy revealed small shrunken kidneys with medullary cysts ranging in size up to 5 mm in diameter

The diagnosis of cystic disease of the renal medulla as well as the difficulty in differentiating this disease from familial juvenile nephronophthisis is discussed It is suggested that cystic disease of the renal medulla and familial juvenile nephronophthisis are probably the same disease the former name being used for cases with gross cysts the latter for those with known heredity

In 1945 Smith and Graham (15) reported the occurrence of numerous small cysts in the medulla of both kidneys of an 8 year-old girl who died in uraemia The patient had had refractory anaemia uraemia isosthenuria but no proteinuria and the urinary sediment had been normal Four similar cases were later reported by Hogness and Burnell in 1954 (5) and in 1962 Strauss (16) reported altogether 18 cases some from the literature and some of his own Several of these cases had been described as salt losing nephritis (10 12 19 20) In 1963 Kerlan et al (9) reported a case in a 42 year-old man and in 1964 Faigel (2) described such a case in a 14-year old boy This paper is concerned with a case of the disease in question and its possible kinship with other renal diseases

CASE REPORT

E. J male born in 1913 His mother is said to have died in uraemia at 35 years of age Necropsy was not done His sister had died in childhood and his three brothers are still alive and healthy A male cousin was hospitalized in 1966 with uraemia and this patient s brother had moderate renal insufficiency

In 1963 the patient complained of increasing weakness, polyuria nycturia and numbness of the legs with cramps of the calves He also reported headache nausea and occasionally also vomiting In December 1963 he rapidly became worse—probably in association with acute gastroenteritis—and in the course of a few weeks during which he often vomited he lost about 10 kg body weight his general condition deteriorated and he was admitted to hospital with muscle cramps

He was pale somnolent and had fine muscular twitching B P 160/90 mm Hg ECG showed no left sided preponderance Eye ground changes grade 2 (K W) Roentgen examination showed the heart to be of normal size Roentgenographic size of the kidneys right 10 × 4 cm, left 10 × 5 cm Roentgen examination of the skeleton revealed nothing remarkable Serum creatinine 15 9 mg/100 ml N P N 254 mg/100 ml No acidosis Serum sodium 133 mEq/l Serum chloride 79 mEq/l Serum potassium 3 4 mEq/l Serum calcium 3 9 mEq/l Serum phosphorous 9 1 mg/100 ml Natriuresis 5 9 g/day Kaliuresis 1.5 g/day Hb 9 5–8 3 g/100 ml

Urine Repeated examination revealed protein on one occasion only The urinary sediment was always normal except for a small number of erythrocytes on a few occasions Repeated culture gave no significant growth Specific gravity 1 010–1 011

The patient responded well to treatment with forced intake of fluid and sodium chloride and after six weeks the serum creatinine was 9 8 mg/100 ml The serum sodium and chloride increased only slightly The patient left hospital in January 1964 and did well until April that year when he had a short recurrence of vomiting and required parenteral infusion of fluid Similar episodes occurred in July and September The loss of salt persisted and the serum sodium was low (lowest 118 mEq l) (Fig 1) In August he was given supplementary sodium chloride which was followed by pulmonary congestion and pleural effusion after which it was difficult to control his electrolyte balance He was admitted to hospital for the last time on October 3 and on that occasion uraemia was more severe and obstinate He died in uraemia on November 8

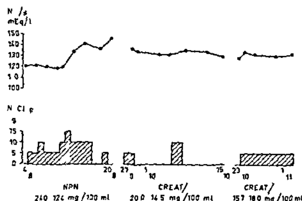


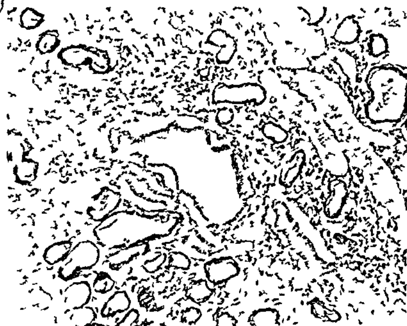
Fig 1 Oral salt intake and serum sodium during three peri ds at hospital in the latter part of the disease


Fig 2


Fig 3

Figs 2 and 3 *Right kidney Atrophic cortex Cysts* of varying size predominantly in medulla

Necropsy was performed five hours after death Th heart weighed 480 g and showed moderate hypertrophy of the left ventricle Several branches of the pulmonary artery cont uned small emboli Both kidneys were dif fusely shrunken and weighed 60 and 65 g The fibrous capsule stripped easily and the surface was reddi h brown nodular and showed no gross cicatricial contrac tions (Fi 2) A few cysts up to 1 cm in diameter w r scattered over the surface The cut surface showed an atrophic cortex 2–4 mm in thickness (Fi 3) Th parenchyma was studded with a large number of cysts varying in ize from that of a pin s head to 5 mm in diameter Most of the cysts and all the large ones were situated in the medulla but some were al o seen in the cortex Thou h num rous the cysts occupied only a small proportion of the cut surface The four para thyroid glands were enlarged and weighed together 1 4 g



Fi 4 Atrophic medullary tubules with thickenin of basement mem brane PAS 1 0

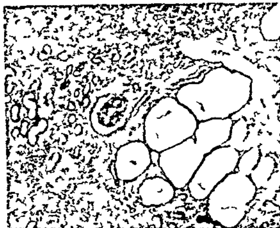


Fig 5 Cort x *Left* Atrophic tubu es and peritubular fibrosis *Right* A group of d lated tubules fusing by ruptures of s ptal wal s H E ×75

One of them contained a cyst 3 mm in diameter and f led with a clear flu d

Histolo cal examination revealed more or less severe destru tion of most of the glomeruli All d rees of damage rangin, from sl ght peri lomerular fibrosis to com lete hyal n ation of glomeruli were seen The pre served glomeruli many of which were hypertrophic had normal cap llary loops without thicken ng of the bas m nt membranes or any epithelial crescents (Fig 7)

Both the cortex and the medulla showed a marked in crease of the *interst tial connective tissue with streaks or* lymphocyt s plasma cells and histiocytes Most of the convoluted and collectin, tubules had disappeared Many of the survi in, tubules were very atroph c and lined by low cub c epithelium S attered hypertrophic tubules with a h h cylindrical ep thel um were found n the cortex as well as in th medulla The h pertrophic tubu es of the me u a showed a characteristic tortuos ty (Fig 6)



Fi 6 Medulla Cysts lined with flattened epithelium A f w tortuous hypertrophic tubules s attered in other wis extensiv ly atrophic tissue van Gi son ×65

PAS-staini, revealed thickened hyalin basem nt mem *brane in the atrophic tubu es (Fig 4) These tubule* were surrounded by connective tissue mantles wh h s ained positiv ly for colla en

Th cys s had probably be n caused by fusion o grou s of c osely packed tubules (F, 5) The small cysts were therefore lined by tubular epith lium Th



F 7 Cortex Atroph tubules with per tubular fibrosis Periglomerular fibrosis H E 100

large cysts on the other hand had a very low endo thelium like epithelium Compression and concentric stratification of the structures adjacent to the cysts suggested that the intracystic pressure was relatively high

Moderate deposits of polymorphonuclear leucocytes were found in the lumina of some of the tubules

The walls of the arteries showed moderate fibrous thickening

The parathyroid glands were built up histologically of chief cells and oxyphilic granular cells

Sections of the vertebral column showed only significant changes of the type seen in fibrous osteoclasia

DISCUSSION

Common to the cases reported by Strauss (16 17) were progressive fatal uraemia little or no proteinuria and isosthenuria In most of the cases the urinary sediment was normal Only one of the patients had hypertension Clinical signs of secondary hyperparathyroidism in the form of inhibited bone growth renal rickets and epiphyseolysis occurred in most of the seven patients between the ages of 8 and 18 years

Since most of Strauss personal cases were clearly salt losing he made a search of the literature for cases of salt losing nephritis Among 16 such cases he found four with small cysts in the renal medulla Later Hughes (7) described a case of salt losing nephritis in which symmetrically shrunken kidneys also contained cysts The initial symptoms such as polyuria and polydipsia had in most cases existed for several years before the first medical examination Since the uraemia progresses so slowly the patients do not seek medical advice until late It is often some complicating and trivial disease in our case acute *gastroenteritis which causes a suddenly life* threatening deterioration in a patient with severe uraemia In several cases the uraemia was discovered accidentally on investigation of refractory anaemia

Of Strauss 18 cases six were females aged 8 to 56 years At follow up the blood urea in an identical twin brother of one of the patients was found to be increased Another patient whose mother was said to have died from Bright s disease had a brother with increased serum urea A renal biopsy specimen from the last mentioned patient which however consisted of tissue only from the cortex showed changes compatible with those seen in cystic disease of the renal medulla Since no heredity was known in the other cases Strauss thought the disease not to be hereditary

The impaired concentrating power of the kidney can be explained by destruction of the tubules in the medulla which first and foremost involves the distal parts of the nephrons Strauss drew attention to the mantles of collagenous connective tissue around the atrophic tubules and collecting tubules he expressed the view that these changes may mechanically prevent the action of a countercurrent multiplier and the large amount of fibrous tissue might inhibit equilibration of tubular fluid with the interstitium

The unrewarding examination of the urinary sediment and the absence of growth on culture of the urine in most of the published cases makes the diagnosis of chronic pyelonephritis less probable The symmetric contraction of the kidneys without gross scars also argues against chronic pyelonephritis The abundant deposit of chronic inflammatory cells need not have been due to an infection for it may have been a manifestation of a response to extensive severe tissue destruction

Unlike *polycystic kidneys* kidneys with cystic disease of the medulla are always abnormally small and the cysts show a predilection for the medulla The clinical picture of polycystic kidneys is quite different Pain haematuria and hypertension are common symptoms while loss of salt is extremely rare

In *medullary sponge kidney* there is cystic dilatation of the collecting tubules in the papillary apices The changes are often asymmetrical and may affect only one kidney or only some papillae of one kidney The cysts often show calcifications and squamous epithelial metaplasia of their epithelium *Patients with medullary sponge kidney* often have local symptoms of renal stone haematuria and urinary tract infections but they rarely have renal insufficiency leading to uraemia

Our case showed all transitional forms ranging from cystic dilatation of the tubules and cysts due to rupture of adjacent tubular loops to gross cysts The largest cysts were situated near the papillary apices and may have been dilated shut off segments of the collecting tubules

CYSTIC DISEASE OF THE RENAL MEDULLA AND FAMILIAL JUVENILE NEPHRONOPHTHISIS

Cystic disease of the renal medulla resembles familial juvenile nephronophthisis a disease first

described by Fanconi et al in 1951 (3) which had apparently passed unnoticed in USA until 1964 (11) In Sweden cases have been described by Hackzell et al (4) von Sydow et al (18) and Broberger et al (1) There is at most temporary proteinuria the urine is sterile and hypertension does not develop Both diseases lead to uraemia They differ from one another in that juvenile nephronophthisis starts in childhood is clearly hereditary and as a rule the kidneys are not cystic

Of seven cases of juvenile nephronophthisis described by Broberger et al (1) the kidneys in two—both 12 year-old children—were thoroughly examined by Ivemark et al (8) These patients were older than those previously autopsied The kidneys were abnormally small in both cases and the cortex was severely atrophic while the medulla was of normal thickness The cysts were situated in the medulla Histologically many glomeruli had degenerated into hyaline balls while others showed various degrees of periglomerular fibrosis The capillary loops of the latter were preserved Several glomeruli were hypertrophic In most cases the tubules were severely atrophic Some tubules were however hypertrophic and their Henle s loops were markedly twisted in a characteristic way The descending limbs of Henle s loops were three times as long as normal The tortuosities were therefore ascribed to hypertrophy of the tubules and not to shrinkage of the kidney The cysts were found to be in open communication with tubules usually Henle s loop or were dilated shut off segments of the collecting tubes

The oldest patient with juvenile nephronophthisis was reported by Holmgren (6) The patient was a 23 year old woman who had salt losing nephritis and no known heredity for renal disease and whose symmetrically contracted kidneys contained numerous gross cysts in the medulla This case would fit in better in the group which Strauss called cystic disease of the renal medulla But nephronophthisis can occur in adult age as shown by the sibship in which three members died from the disease at 4 10 and 21 years of age (14)

It is possible that the disease is more protracted in adults If the disease develops slowly more nephrons would have time to hypertrophy and in a later stage of the disease those hypertrophied nephrons are obstructed by the contracting process and developing cysts In very young children on the other hand it may be assumed that the disease runs a more rapid course and that most of the nephrons are affected at the same time This would explain why cysts are more often seen in adults

Symptoms of salt losing nephritis were common in Strauss series and have been described mostly in elderly patients with nephronophthisis It might be of interest to analyse the published cases of salt losing nephritis which Strauss did not include in the group of cystic disease of the renal medulla in order to find cases resembling nephronophthisis Only a few of the patients have however been autopsied Of the cases examined post mortem several were found to have nephrocalcinosis while others with contracted kidneys were diagnosed as chronic pyelonephritis But in one case described by Nussbaum et al (13) there was heredity for renal disease and renal lesions compatible with the diagnosis of nephronophthisis This was a 37 year old woman who died of salt losing nephritis Her brother and father had died of renal disease She had no arterial hypertension or albuminuria and the urinary sediment was normal The shrunken kidneys showed histological changes which were interpreted as chronic pyelonephritis but the changes described are also compatible with nephronophthisis

The heredity of familial juvenile nephronophthisis appears to be autosomally recessive It might therefore also sometimes occur sporadically The absence of relatives with renal disease in some cases of cystic disease of the renal medulla would not exclude such a hereditary mechanism In this connection the familial cases of renal disease in Strauss series are of interest and particularly the pair of identical twins of whom the surviving brother was found to have uraemia Strauss thought that it could be explained by a developmental anomaly or an exogenous factor common to both In the light of the similarities between the disease and juvenile nephronophthisis the hypothesis of a hereditary mechanism seems more likely

There appears to be strong reason to assume that cases described under the name of juvenile nephronophthisis and cystic disease of the renal medulla belong to one and the same clinical entity Cases of the disease without known heredity

or cysts in the kidneys have probably been formerly diagnosed as chronic pyelonephritis This resulted in an artificial differentiation cases with renal cysts being published under the name of cystic disease of the renal medulla and cases with known heredity under the name of juvenile nephronophthisis

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COMPLIANCE IN BRONCHIAL ASTHMA

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Abstract In a series of patients with bronchial asthma studied in free intervals the lung compliance was deter mined both on quiet spontaneous respiration and on respiration at a frequency of 40 per minute The effect of bronchodilatation (inhalation of isopropyl noradrena line sulphate) on compliance was also stud ed

The compliance values on quiet respiration were distributed both w thin and outside (mainly below) the limits previously reported as normal Hyperventilation caused a reduction of lung compliance Bronchodilatation gave an increase of compliance both on spontaneous respiration and on hyperventilation

Correlation calculations were performed in order to study the relationship between compliance and certain case history data or the results of different lung function tests

The mechanics of respiration in chronic obstructive pulmonary disease have been studied by several investi ators The conditions both in bronchial asthma and in pulmonary emphysema have been studied (for review see Ehrner (2)) and in these series a considerable proportion of the patients have shown compliance values within the normal range The scatter is great however and especially in emphysema patients considerably raised as well as considerably lowered values of compliance have been recorded

Only to a limited extent have patients with uncomplicated bronchial asthma been subjected to studies of pulmonary mechanics On analysis of series of asthma patients studied in this respect it is often found that to a large extent the asthma has become complicated by chronic bronchitis or that chronic bronchitis has been predominant ever since the onset of the asthma Furthermore the investigations often have not been carried out in so-called free intervals i e the patient has been in an optimal condition when from the point of view of his asthma Out of 148 cases with various chest diseases Ehrner (2) reported only three with uncomplicated bronchial asthma studied during free intervals and in these three patients he found normal compliance values during both spontan ous breathing and hyperventilation

Thus there seems to be no report in the litera ture concerning respiratory mechanics in a large and uniform group of asthma patients The aim of the present investigation therefore was to study firstly the dynamic pulmonary compliance during both spontaneous and forced breathing and sec ondly the effects on the compliance of broncho dilatation produced by isoprenaline inhalation The study was performed in patients with un complicated bronchial asthma during free inter vals The compliance values and isoprenaline ef fects were correlated to certain background data from the case history such as the age at onset of the asthma the assessed degree of severity of the asthma and its duration and also to the values for lung volumes ventilatory capacity and phys ical work capacity

MATERIAL

The material consisted of 69 patients (9 men and 40 women) with bronchial asthma None of the pat nts ful fil ed the criteria for ch onic bronch tis The principles followed for d fferentiation between the different chronic obstructive pulmonary diseases were those suggested by the American Thoracic Society Table I shows the com pos tion of the material with regard to certain case history data, and to the ventilatory capacity lung vol umes and phys cal work capacity The degree of se erity of the asthma was assessed by means of so-called sick ness points—for def nition see Irnell (5)—both for the total period since the onset of the asthma and for the f e year per od immed ately preced ng the investigation The majority of the patients were assessed as having very se ere or severe asthma, and a small number moderately se ere asthma The a erage age of the patients at the time of investigation was 5 years, and their average age at the onset of asthma was just o er 3 years

Table I *Mean values and standard deviation for certain case history factors and function variables*

Factor	*n*		Mean value			S D		
	Men	Women	Men	Women	Men+ women	Men	Women	Men+ women
X_1 Age y	29	40	53 6	54 8	54 3	13	10	11
Y_2 Total sickness points since onset of asthma	29	40	1474 5	1807 1	1668 1	959	1267	1152
X_3 Mean annual sickness points for last 5 y	28	40	48 1	54 7	52 0	50	40	44
X_4 Age at onset y	29	40	32 6	33 0	32 8	19	15	17
X_5 Duration of asthma y	29	40	20 4	20 6	20 5	10	11	11
X_6 VC of pred	29	40	84 6	87 2	86 1	16	13	14
X $FEV_{1\,0}$ of pred	29	40	72 0	71 3	71 6	24	18	20
X_8 FEV of pred	29	40	81 9	79 7	80 6	19	15	17
X_9 MVV_F of pred	29	40	61 5	58 1	59 5	21	19	20
X_1 FRC/TLC of pred	29	38	111 2	121 9	117 3	13	17	16
X_{11} RV/TLC of pred	29	38	138 4	145 8	142 6	24	29	27
X_2 W_{13} of pred	28	40	81 0	77 3	78 8	27	31	29
X_{13} W max kpm/min	28	40	781 8	446 1	583 3	280	141	266
X_{14} W max of pred	28	40	84 8	81 4	82 8	25	25	25

Of the 69 patients five men and five women also had apart from their bronchial asthma a chronic disease the possible influence of which on the cardiopulmonary function was somewhat uncertain The results for these ten patients are therefore not included among those for the main group of patients but are given separately Of the 59 patients who thus constituted the main material those below the age of 60 years also underwent right heart catheterization Neither at this nor at other investigations e g electrocardiography was any primary or secondary cardiac disease or dysfunction disclosed

METHODS

All tests were performed during free intervals which meant that during the test period and for at least two days immediately preceding the test the patient was either completely symptom free or showed a subjective freedom from symptoms that had not been surpassed at any other time during the previous 12 months Before a test was carried out it was ascertained that no rhonchi were heard on auscultation of the lungs during normal breathing

The methods for measuring lung volumes ventilatory capacity and physical work capacity and also those for haemodynamic studies have been described previously (5) The international nomenclature for respiratory data is used

The dynamic lung compliance was calculated in litres per cm H O as the ratio $\Delta V/\Delta P$ where ΔV is the tidal volume and ΔP the intraoesophageal pressure change corresponding to the tidal volume from the end of an expiration to the end of an inspiration i e measured between two points at which the air flow at the mouth was zero The tests were performed in the morning, the patient having fasted since the previous evening The tests were carried out in the upright sitting position

For *determination* of the intraoesophageal pressure a duodenal catheter (no 16 Mediplast) was introduced nasally Local anaesthesia with Xylocain® (Astra) was administered both as a nasal spray and as a throat gargle The catheter was lubricated with Xylocain gel to facilitate insertion Attached to the lower end of the catheter was a narrow inflatable balloon 12–14 cm long (Nordiska Latexfabriken Torekov Sweden) The balloon was filled with 1–1 5 ml air and the distance from the nasal opening was varied until a good pressure recording was obtained with the least possible disturbance from the heart contractions The catheter was then fixed to the cheek For determination of the pressure difference between the oesophagus and the mouth the upper end of the catheter was connected to a differential pressure *manometer (EMT 490 Bd (Elema Schonander Solna* Sweden) linear between 0 and 30 mm Hg) with an amplifier (EMT 460 type Q 799 (Elema Schonander Solna Sweden))

For the flow and volume recordings the patient breathed through a pneumotachograph (A Fleisch) which was connected to a differential pressure manometer (EMT 572 (Elema Schonander Solna Sweden) for pressure measurements between 0 and 50 mm H O) The flow meter had a practically linear response for flows up to 3 l/sec (as stated by the manufacturer) It was used here to measure the respiratory volume by electrical integration and to establish the zero points in the air flow The amplifier used was an EMT 573 (Elema Schonander Solna Sweden) (time constant 10 or 20 sec)

The two pressure transducers were of the inductive type A Mingograf 42 B (Elema Schonander Solna Sweden) was used throughout for further amplification and writing

For volume calibration of the apparatus one of the meters in a bronchospirometer (Kifa Solna Sweden) was used connected serially to the pneumotachograph Such a connection was also used during certain investigations on the patients to enable a check to be made of

the tidal volume as obtained from the electrical integrator Such a check was necessary since the integrator is to some extent frequency dependent and gives a rather small deflection with increasing frequency Further with the electrical integration the volume obtained *during* a respiratory movement is somewhat below the true value For this reason the values of the air flow resistance are not reported here since the change in volume could not be precisely measured from the commencement of inspiration to the time when a given air flow rate had been reached the air flow resistance should be given at a definite flow rate (e g 0.5 or 1 0 l/sec) to allow intra *and interindividual comparisons to be made*

The compliance was calculated both before and after bronchodilatation with isopropyl noradrenaline sulphate nebulized in a Medihaler (manufactured by R ker Lab Inc Loughborough England) Each time the medihaler is released 0 06 mg isoprenaline should be nebulized The average particle size should be 2 1 μ The patient was instructed to breathe out deeply and then immediately—just at the start of a deep inspiration—to inhale a medihaler dose of isoprenaline At the end of the inspiration the patient held his breath for about three seconds in order to obtain an optimal effect of the inhalation Two such inhalations were performed at an interval of one minute The patient was then asked to try to clear his airways by coughing and clearing his throat Ten minutes after the second inhalation the second compliance determination was commenced

For the statistical analyses the same methods were used as were reported by Irnell (5) The following degrees of significance were employed almost significant ( ) if $0\ 01 < P < 0\ 05$ significant ( ) if $0\ 001 < P \leqslant 0\ 01$ highly significant ( ) if $0\ 001 \geqslant P$

Statistical advisor Gunnar Eklund Ph D

RESULTS

Figs 1 and 2 show the distribution of the patients with regard to lung compliance (C_L) before bronchodilatation with isoprenaline during quiet spontaneous respiration and during rapid respiration (40/min) respectively

To obtain the value by which the patient is represented in the figures the following procedure was used For each patient five compliance values were obtained first during quiet respiration with and without a spirometer and then during rapid respiration with and without a spirometer For each of these conditions the highest and lowest values were excluded and the mean value of the remaining three was then calculated Measurements were performed both before and after isoprenaline inhalation

The difference between the compliance without and with the spirometer was on average ($\pm$ standard error of mean (S E)) as follows

On quiet respiration before isoprenaline inhalation	$0\ 007 \pm 0\ 012$ ($n = 6?$)
On rapid respiration before isoprenaline inhalation	$-0\ 005 \pm 0\ 007$ ($n = 43$)
On quiet respiration after isoprenaline inhalation	$-0\ 014 \pm 0\ 016$ ($n = 61$)
On rapid respiration after isoprenaline inhalation	$0\ 007 \pm 0\ 007$ ($n = 4\ $)

In no case was the difference significant and the very small differences noted showed no uniform tendency It was therefore considered justifiable for presentation of the results to combine the compliance values obtained with and without the spirometer Each patient is thus represented by such combined values in Figs 1 and 2

From the figures the compliance appears on the average to be higher on quiet than on rapid respiration Statistical analysis of the individual values showed that this was in fact so the mean difference $\pm$ S E for compliance (quiet respiration minus rapid respiration) being $0\ 137 \pm 0\ 019$ (65 comparisons) This difference is highly significant (***) *The corresponding difference for the*

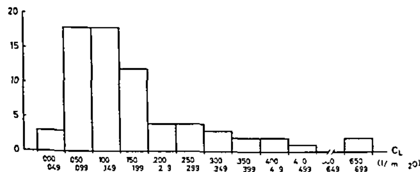


Fig 1 Distribution of patients with regard to lung compliance on quiet respiration before isoprenaline administration

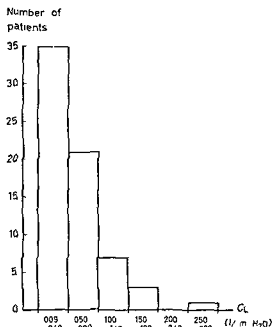


Fig ? Distribution of patients with regard to lung compliance on rapid respiration (40/min) before isoprenaline administration

values after bronchodilatation with isoprenaline was 0 125±0 018 (65 comparisons) This difference was also highly significant (***)

Table II (left part) shows the coefficients of correlation with regard to the relationship between on the one hand compliance during quiet respiration before isoprenaline inhalation and on the other certain case history factors and function variables These coefficients were calculated for the 59 patients with uncomplicated bronchial asthma It can be seen from the table that with the exception of the age at onset in the women and $FEV_{1\,0}$ in the men there was no statistical correlation between the compliance value on quiet respiration and the selected factors

For these patients with uncomplicated asthma an analysis was performed with the aim of ascertaining whether the isoprenaline effect showed correlation with the background data (sickness points for assessment of the degree of severity of the asthma) and with the values of ventilatory capacity lung volumes and physical work capacity in the present investigation For this analysis the effect of isoprenaline on the

Table II

Left Coefficients of correlation with regard to relationship between (*a*) lung compliance on quiet respiration before isoprenaline administration and (*b*) certain case history factors and function variables
Right Coefficients of correlation between (*a*) effect of isoprenaline on compliance (defined as a quotient—see text) on quiet and on rapid (40/min) respiration and (*b*) certain case history factors and function variables

	Coefficients of correlation					
	Compliance quiet respiration before isoprenaline		Compliance quotient			
			Quiet respiration		Rapid respiration	
Factor	*Men* 23 *n* 24	*Women* 34 *n* 35	*Men* 23 *n* 24	*Women* 31 *n* 3	*Men* 20 *n* ₋1	*Women* 32 *n* 33
X_1 Age y	0 06	−0 33	0 26	0 16	0 14	0 ?9
X_2 Total sickness points since onset of asthma	−0 26	0 02	−0 06	0 19	0 08	−0 0?
X_3 Mean annual sickness points for last ⁵ y	−0 10	−0 22	0 02	0 25	−0 05	−0 08
X Age at onset y	0 20	−0 47 *	0 31	−0 02	0 13	0 08
X_5 Duration of asthma y	−0 35	0 23	−0 25	0 17	−0 08	0 13
X_6 VC of pred	0 36	0 30	−0 23	−0 18	0 25	0 ?0
X_7 $FEV_{1\,0}$ of pred	0 42	0 13	−0 24	−0 12	0 14	0 10
X_8 FEV∞ of pred	0 40	−0 04	−0 26	−0 27	0 07	−0 02
X_9 MVV_F of pred	0 30	0 25	−0 17	−0 23	0 10	−0 10
X_{10} FRC/TLC of pred	−0 09	−0 06	0 06	0 25	0 01	0 04
X_{11} RV/TLC / of pred	−0 20	−0 18	0 04	0 35	−0 29	0 15
X_{12} W_{130} of pred	0 19	−0 02	−0 13	−0 ₊5	−0 31	−0 07
X_{13} W max kpm/min	0 13	0 20	−0 05	−0 01	−0 26	−0 10
X_{14} W max of pred	0 24	−0 12	0 15	0 17	−0 24	0 22
Sign level 95 confidence	0 40	0 33	0 40	0 35	0 42	0 34

Table III *Effect of isoprenaline inhalation on compliance value (in l/cm H_2O) both on quiet respiration and on rapid respiration (40/min)*

The effect is expressed as the difference between the compliance value obtained after and that obtained before i oprenaline Mean (*M*) standard deviation (S D) and standard error of mean (S E) for the differences

Patient group	Quiet respiration *n*	*M*	S D	S E	Rapid respiration *n*	*M*	S D	S E
Patients with bronchial asthma alone	59	0 041	0 110	0 014	57	0 028	0 046	0 006
Patients w th other disease apart from bronchial asthma	10	0 043	0 054	0 017	9	0 003	0 038	0 013

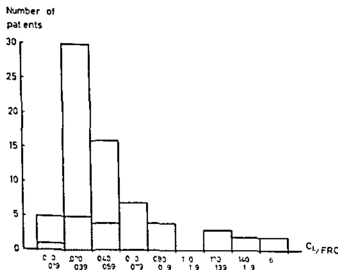
compliance was given for each patient as a quotient viz

$$\frac{\text{compliance value after isoprenaline} - \text{compliance value before isoprenaline}}{\text{compliance value before isoprenaline}}$$

The result of the correlation analysis is given in Table II (right side) It was found that the co f ficient of correlation for RV/TLC for women lay at the level of statistical significance The sig nificance level was not reached however when the means of the coefficients of correlation were obtained for men and women combined None of the other coefficients of correlation reached the significance level The factors which in the table show the highest correlation (when the coef ficients were combined row by row) were age (X_1) and W_{130} (X_1) This su gests that with in creasing age a stronger isoprenaline effect is ob tained and that a high value for W_{130} is accom panied by a low isoprenaline effect

The effects on compliance of bronchodilatation with isoprenalin on quiet and on rapid respira tion respectively are shown in Table III This table shows that on quiet respiration the com pliance value increased after isoprenaline ad ministration by an average of 0 041 l/cm H O The corresponding value on rapid respiration was 0 028 l/cm H O Both on quiet and on rapid respiration 43 patients showed a positive dif ference (higher compliance value after isoprena line administration) while 16 patients showed a negative difference

The ten patients who had another disease apart from their bronchial asthma are reported separa tely in Table III These patients showed similar tendencies to those of the larger group On rapid respiration however the mean effect was very small



F g 3 D tr b t on of patients acco d n to alues for lun compl ance FRC The total he ht of the bars rep e nt the number of pat ents in e h clas and the cro s-hathed pa ts represent the number of p tient with n th la from the g oup w th anoth r d eas apart f m b n h l a thma The lowe t alue 0 000–0 019 w 0 008 The tw alues n cl 0 160 d ho e we e 0 15 and 0 311

Table IV *Coefficients of correlation with regard to relationship between (a) the quotient C_L/FRC and (b) age at onset and certain lung function variables*

Factor	Coefficients of correlation: All patients, Men n = 29	All patients, Women n = 40	Patients with bronchial asthma alone, Men n = 24	Patients with bronchial asthma alone, Women n = 35
X_4 Age at onset y	0 19	−0 33	0 20	−0 41*
X_6 VC of pred	0 32	0 35	0 35	0 32
X_7 $FEV_{1.0}$ of pred	0 47	0 23	0 47	0 20
X_8 MVV_F of pred	0 40	0 35	0 39	0 32
Sign level 95 confidence	0 36	0 31	0 40	0 33

A number of previous investigators have demonstrated a relationship between on the one hand compliance and on the other lung volumes and body height (2 4 7 10) In order to eliminate the influence on the compliance value of differences in body size and lung volumes among the present series of patients a specific compliance value (8) was calculated as the quotient C_L/FRC for each individual patient The distribution of these values among the series of patients is shown in Fig 3 The statistical correlation between this quotient and certain variables is shown in Table IV It can be seen from the table that for the total material there is now a correlation between reduced maximal voluntary ventilation and reduced compliance The corresponding values for the group with uncomplicated bronchial asthma lie near to the significance level

DISCUSSION

Lung compliance

In approximately half of the patients the value for lung compliance on quiet respiration was found to lie within the limits reported by other authors as normal i e 0 10–0 30 l/cm H_2O (1 2) In most of the remaining patients this value lay below the lower normal limit

During an asthma attack reduced values may be recorded which may become completely or partly normal again when the attack is over (3 11) Residual reduced values have been reported in the literature and were also noted among the present patients which indicates that in a number of cases restitution is not complete This also seems reasonable in view of the fact that the functional ventilatory capacity was usually reduced in our patients and it might be expected that this will also be manifested as a change in lung compliance It is true that no statistically significant relationship was found between lung compliance and the lung function variables selected but the covariance was fairly good When the quotient C_L/FRC was used instead the covariance improved further and for certain factors it almost reached the significance level

The difference between the compliance value on quiet and rapid respiration was highly significant This is not an ordinary finding in normal individuals when the respiratory frequency is moderately increased but was probably due to abnormal air flow resistance and/or abnormal intrapulmonary gas distribution with reduced ventilated lung volume and altered elastic properties (9) In his mixed series—of which however only a small number of the patients had an obstructive pulmonary disease—Ehrner (2) appears to have found a considerably smaller reduction of the compliance value on hyperventilation than that shown by our series On quiet respiration however Ehrner s patients showed on the average a lower compliance than our patients This may simply be due to the difference in the disease composition of the two series of patients In contrast to most series reported in the literature our series consisted of patients with uncomplicated bronchial asthma and no absolute comparisons can therefore be made There had of course been periodic infections among the patients of our series and these may have given rise to destruction and changes resulting in reduced compliance but in no case was for example chronic bronchitis present

The statistical correlation found between age at onset of the asthma in women and compliance (Table II) was probably not a true one Biologically there seems to be no reason to suppose such a relationship which is further contradicted by the fact that the corresponding coefficient of correlation for men was of the opposite sign and of considerable numerical value

Effect of isoprenaline

Significant changes in lung compliance occurred after bronchodilatation both on quiet and on rapid respiration These changes however showed no correlation with the background data or function variables studied It is possible that a significant relationship in this respect might have been found if the different function variables had also been subjected to the isoprenaline test

The reason for the recorded effect of isoprenaline on compliance on quiet respiration was probably a change in distribution of the tidal volume with ventilation of a larger lung volume which increases compliance and also possibly a change in the mean respiratory level The isoprenaline effect on rapid respiration should furthermore have concerned those factors which play a part in high respiratory frequency (see above) The flow resistance in the airways was not studied in this investigation but the changes of compliance during rapid respiration as well as the effect of isoprenaline give indirect information in this respect Also supporting the probability of a reduced air flow resistance after isoprenaline inhalation in these patients are the improved achievements in dynamic lung function tests which have been studied separately (6)

ACKNOWLEDGEMENT

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Universite de Paris Faculte de Medecine *Les Journees Medicales Annuelles* de l Hopital Broussais La Charite sous la Presidence du Professeur Pasteur Vallery Radot de l Academie Française Service du Professeur Paul Milliez auront lieu jeudi 9 vendredi 10 et samedi 11 mai 1968

Il est recommande de s inscrire assez tot le nombre des participants etant limite Priere d envoyer les droits d inscription au Centre de Recherches sur l Hypertension Arterielle Professeur Milliez Hopital Broussais 96 Rue Didot Paris 14 (cheque bancaire ou mandat carte) Les droits d inscription sont de 100 F tout compris (ensemble de ces journees et volume des conferences) Un ticket de reduction SNCF sera adresse sur demande

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Principal theme Modern aspects in nutrition of individuals and populations (clinical aspects technology) Languages English (French German Russian)

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SUPPRESSION OF ECTOPIC BEATS BY AJMALINE IN A PATIENT WITH ARTIFICIAL HEART PACEMAKER

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Abstract In a 73 year-old woman with an implanted heart pacemaker interpolated ectopic ventricular beats pro oked syncopal attacks The paraelectrical excitations could be stopped completely by the administration of ajmaline either intravenously or intramuscularly

Hitherto the main clinical interest in the Rau wolfia alkaloids has been for reserpine How ever Siddiqui and Siddiqui (8) isolated ajmaline in 1932 and as early as 1939 it was evident from the investigation of Bijlsma and van Dongen (1) that this alkaloid was a potent antiarrhythmic agent In 1959 Kleinsorge (2) introduced ajmaline into the therapy of disturbed heart rhythm This drug was found to be most effective in inhibiting ventricular ectopic activity (5 6) Kleinsorge and Volker (4) showed that when normal heart rhythm was restored after bi or trigeminy cardiac output could increase by 44 Ajmaline is rap idly excreted in the urine (3)

CASE REPORT

A 73 year old woman with an implantable pacemaker was admitted to the medical department on July 7 1965 complaining of perpetual short syncopal attacks

In Aug 1959 ECG had revealed a f rst degree A V heart block A chest X ray showed that the heart size was sl ghtly increased The B P was 135/85 mm Hg In the autumn of the same year the pat ent had her f rst Stokes-Adams seizures and the ECG showed a complete A V block She was placed on atropine and ephedrine b mouth In February 1960 lanatoside C potassium and a thiazide d uretic were added to the regimen During the following years she was troubled only by negligible syncopal ttacks periodically But in April 1963 she suffered se eral severe Stokes Adams seizures which con tinued during May Therefore on June 7 1963 an in ternal pacemaker (Elema Schonander) was implanted with the electrodes attached to the left ventricular wall close to the apex (Professor V O Bjork, Uppsala) Atrop ne ephedrine and d gitalis were d scontinued Sub sequently she was on bend oflumeth azide 2.5 mg potassium chloride 0.57 g and amitriptyline 10 mg daily A chest X ray on June 3 1964 showed the total heart volume to be 13 5 ml corresponding to 750 ml/m of body surface

The pacemaker unit was replaced on July 28 1964 and on June 23 1965 Five days after the last pace maker exchange the patient was placed on digoxine 0 25 mg daily In the eight-day period following the adminis trat on of this digitalis preparat on she was troubled by increasing dizziness irregular heart activity and syncope

Examination on admission

The pat ent was found to be anxious and pale Her l ps were slightly cyanotic Blood pressure was 170/90 mm Hg When auscultating the heart one had the impression that two ectopic ventricular contractions appeared after the pacemaker induced beats As the radial pulse rate was about 35 and the pacemaker adjusted to produce 70 impulses/min it was presumed that the heart only responded to every second electrical stimulus and that the ectopic beats (EB) were unable to bring about any significant hemodynamic effect.

However ECG showed that the heart ventricles were excited by each pacemaker impulse But almost every *second regular contraction was followed by an EB* (Fig 1 A) It was also noticed that at least four types of EB occurred (Fig 1 B) Now and then short periods of bigeminal EB appeared and on such occasions the pa tient was always on the verge of syncope (Fig 1 C)

D goxine was discontinued and the patient was sub sequently under close observation The cond tion was unchanged during the next 4 hours As the extrasystoles arose from numerous centers they obviously constituted precursors of life-endangering ventricular tachycardia Among various means of eliminating this risk and of reliev ng the patient from the syncopal attacks, a deci s on was finally made to try ajmaline

Procedu e

The pat ent was brought to the emergency room and connected to a Mingograf Card rex 4 B Ten ml of a 0 5% solution of ajmal ne (Gilurytmal® ad intravenosum produ ed by G ul ni Ludwigshafen Rhein) was drawn into a yr nge It w as umed to be fairly safe if one ml (5 mg) was nje ted intra enously during ten to f ftee seconds at ntervals of one m nute

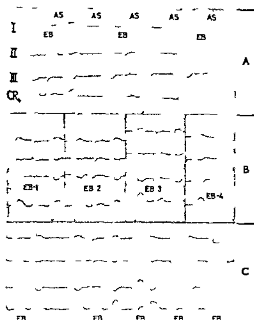


Fig 1 ECG from the patient at admission. *AS* artificial stimulation *EB* ectopic beat Generally every second AS was followed by an EB (A) Different types of EB (EB 1 EB 2 etc) appeared (B) During certain periods every AS was followed by an EB (C)

B P was recorded sphygmomanometrically and the radial pulse rate examined digitally

When the ECG was started the rather monotonous picture from the preceding day was seen on the strip

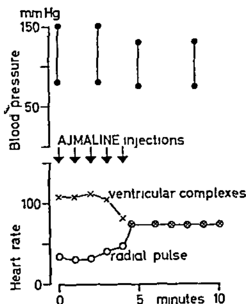


Fig 2 The change in the hemodynamic conditions during the administration of ajmaline At each arrow 5 mg of ajmaline was given intravenously *Ventricular complexes* indicate the sum of the artificially induced beats *and* the extrasystoles

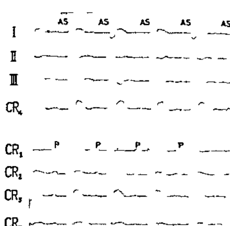


Fig 3 ECG *after* the restoration of the "pure" pacemaker rhythm by ajmaline All the ectopic activity has disappeared In CR 1 and CR 2 the P waves can clearly be seen Thus the complete A V block remained unaffected and the atrial frequency was apparently unchanged

i e interpolated ectopic beats either between every second pair of pacemaker excitations or occasionally in salvoes of bigeminal arrangement. Following 15 mg of ajmaline a decrease of the ectopic excitability could be discerned The E B appeared to begin with in succession to every third regular beat Gradually a change of the pattern of the types of EB was also noticed. From now on predominantly EB-4 appeared and with some modification in shape became more and more attached to the next regular beat After 4 / min when 25 mg of ajmaline had been given all ectopic activity suddenly disappeared Coincidently the radial pulse rate rose to the pacemaker frequency and the patient became alert (Fig 2)

However back in the ward the symptoms returned two hours later and ECG showed that the ectopic rhythm had developed again Now 50 mg of ajmaline (2 ml of a 2 5 solution intramuscularly) was given and some thirty minutes later no EB could be detected Henceforth no ectopic arrhythmia reappeared (Fig. 3)

Later the patient received light sedatives a polythiazide and dipyridamol She could be discharged in good condition after two weeks in hospital

A check up three months later showed that she felt well and the heart was strictly conducted by the artificial pacemaker

Comment

In a 73 year-old woman with an implanted pacemaker *interpolated ectopic ventricular* beats provoked syncopal attacks A recently introduced antiarrhythmic agent ajmaline was found to be able to abolish the ectopic activity without *disturbing the pacemaker in conducting* the heart

a moderate fall in blood pressure was noticed after administration of 25 mg ajmaline intra nously

DISCUSSION

The cause of the ectopic activity in the present ent was not apparent. As digitalis had been ntroduced coincidently with the appearance of the extrasystoles a causal connection was sus However the digoxine dose was not of an which would be expected to produce toxic f . But some factors could have favored an ncreased sensitivity to digitalis e g the presence f the total A V block and the enlarged heart.

It has been shown that ajmaline lengthens the y period more than it reduces the speed conduction (7) This may be the explanation f the prompt effect in the present case Other ythmic agents like quinidine or procain de would probably also have restrained the ctopic activity But theoretically these substances v involve a greater risk of deranging the ac on of the pacemaker

The impulses from the pacemaker must be adjusted to exceed the threshold for effective timulation On the other hand the signals should e well below the threshold for ventricular fibril Ectopic beats decrease the coronary blood ow in varying d grees As a consequence the ocardial ischemia lowers the threshold for fibrillation An antiarrhythmic agent ed in this situation must be able to uppress the ectopic beats without markedly sing the threshold for effective stimulation. ne obviously fulfils these requirements

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THE BLOOD PRESSURE IN A REPRESENTATIVE POPULATION SAMPLE

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From the Bergen Blood Pressure Committee Be gen Norway

Abstract A random sample of the population of Bergen Norway was examined in 1963/64 for blood pressure weight and height in association with a chest X ray survey The sample was made up of all individuals born on the 3rd, 13th and 23rd day of each month from 1864 to 1949 representing approximately 10 of the popula tion 14 years of age and above resident in Bergen on July 1 1963 The study group comprised 845_ subjects 3718 males and 4734 females Of these 8?94 (98 1%) were actually examined 7499 in survey centres and 795 in their private homes. The blood pressure measurements were carried out with a standardized technique by 19 specially trained and supervised nurses The results have been analysed in detail with regard to observer variation For each subject, two sphygmomanometers were used in alternating sequence the cuff was 40 mm long and 14 cm wide

The results of the study are presented in seven figures and two tables details are to be found in ten appendices

The systolic blood pressure was found to vary with sex and age but very l ttle with height weight and pulse rate In females the mean values varied from 118 mm Hg in the age group _0–?9 years to 171 mm at 70 79 years In males, the mean values varied from 1 8 mm Hg at ages 0–?9 years to 157 mm in the 40-49 year group In the two diastolic pressures (phase 4 and phase 5) the same type of sex differences were present but to a smaller degree In females the mean values for the fifth phase increased from 66 to 8_ mm Hg and in males from 69 to 8_ mm The systolic and diastolic blood pressures were well correlated in all age groups of both sexes

The subjects examined in their private homes showed sl ghtly higher blood pressure values than those attending the survey centres The reason for this difference was in- gated but no definite explanation was found

sive knowledge has been accumulated from ous studies on blood pressure conditions in _ population groups and surprisingly good dance has been demonstrated between the lts from different geographic areas Most of the studies have shown that age influences blood pressure values (1 2 11 14) A gradual increase in blood pressure has been found from adoles cence to old age and in females particularly be tween 40 and 50 years of age Furthermore males have demonstrated higher pressures than females in young age groups while in old age females have had the highest values

There has been some correlation of blood pressure with weight skinfold thickness and arm circumference (1 9 14 16) but very little with height (1 9) The correlation with weight and arm circumference has however differed in several studies (7 8 14 16) partly due to varia tions in technical details of the equipment used (10 15) The length and width of the cuff applied to the arm have been significant (10 18)

In the analysis of the population surveys the mean values of the blood pressure in age and sex groups have been used supplemented by the standard deviation In cross sectional as well as in longitudinal studies however the mean values may obscure important details and thus for example assessment of the influence of the age factor may be complicated

The majority of blood pressure surveys have dealt with selected population groups which were not representative of the population from which they were taken Consequently real prevalences have not been obtained and this has affected the general implications of the results The question has constantly been left open What of the blood pressure of the individuals not examined in the surveys? How would the inclusion of an examina tion of these individuals affect the results in particular the mean values?

A priori it may be assumed that the total population results would not differ very much from the results of the surveys of the subgroups

The Bergen Blood Pressure Committee is constituted of from the Univers ty of Bergen School of ed cine Medical Departments A and B and Bergen lelseråd

Table I *Age and sex composition of random sample Bergen population 15 years of age and over*

	Sample			Examined	
Age (y)	Males	Females	Total	No	Per cent
90–99	5	14	19	16	84
80–89	75	130	205	194	95
70–79	254	414	668	644	96
60–69	519	761	1290	1240	97
50–59	618	817	1435	1402	98
40–49	689	764	1453	1404	97
30–39	599	667	1266	1218	96
20–29	608	725	1333	1252	94
15–19	482	482	964	9 4	96
Total	3849	4774	8623	8294	96 2

The justification for this may be found in the great similarity between the findings in different surveys even though the groups actually examined have been selected on a very different basis A number of studies have concerned various kinds of working groups mostly males while others have dealt with subjects invited or selected for reasons such as high tuberculosis prevalence Frequently unemployed subjects particularly housewives have not been included in the studies

Obviously the ideal way of obtaining real prevalence figures and a true picture of the blood pressure conditions in a population would be to examine a large population in total or a representative sample However this has proved to be very difficult In the present study an attempt has been made to meet the ideal requirements by the examination of a representative sample with due consideration of the important technical details in the measurement of the blood pressure

Table II *Age and sex composition of random sample actually present in Bergen during the time of the survey*

	Present			Examined	
Age (y)	Males	Females	Total	No	Per cent
90–99	5	14	19	16	84
80–89	75	130	205	194	95
70–79	253	413	666	644	97
60–69	512	759	1271	1240	98
50–59	610	816	1426	1402	98
40–49	670	762	1432	1404	98
30–39	583	656	1239	1218	98
20–29	556	710	1266	1252	99
15–19	454	474	928	924	99
Total	3718	4734	8452	8,94	98 1

MATERIAL

The sample

In the Blood Pressure Survey of Bergen 1963 arrangements were made to examine B P height and weight of a random sample of approximately 10% of the population of Bergen aged over 14 years (born before January 1 1950) The sample was constituted by all individuals born the 3rd 13th or 23rd day of each month (the digit 3 was chosen at random) among those who were residents of Bergen as per July 1st 1963 The random sample is listed in detail in Tables I and II and is related to the total population of Bergen in age and sex groups in appendix I

During the period of the survey 56 persons died and 179 moved out of the city In addition there were 43 sailors termed residents in the official records of the city even though they had not returned to Bergen for the last 10–20 years and had neither wife nor children, nor room flat or other permanent place of living Furthermore the official records included 21 vagabonds who were assumed absent from the city

In all 299 individuals were excluded from the sample which was consequently slightly smaller than the theoretical size assuming that births are evenly distributed over the days of each month The resulting sample consisted of 8623 subjects 4774 females and 3849 males Of these 171 subjects (39 females and 132 males) were not present in the city during the survey period The majority of these were males on military service (5?) or in the merchant navy (appendix II) Of the subjects present in Bergen in the survey period 8294 or 98 1% were examined

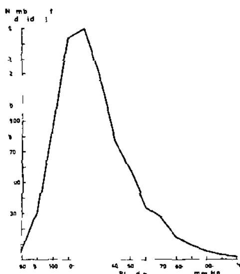


Fig 1 Systolic blood pressure in all age groups, random sample of the population of Bergen 1963

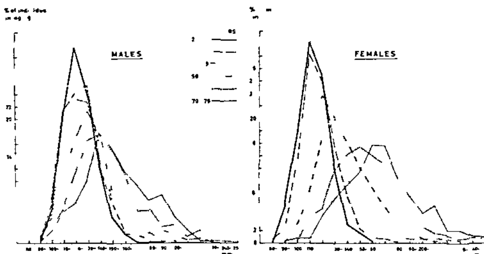


Fig 2 Systolic blood pressure in different age groups in males and females

Some details concerning the 158 subjects who did not take part in the survey may be seen in appendix II Of these 46 had had their B P recently measured in hospital or by their private physician Fourteen had serious disease 13 had difficult conditions of work, etc while 57 (0 7%) of the total sample had no reason for absence from the survey other than simple refusal

Blood pressure examinations

The examinations of B.P., weight and height was carried out in association with a mass radiography chest survey of those of the Bergen population aged 14 years and over In all 7499 of the subjects of the random sample were thus examined In addition 795 subjects who did not attend the survey centres were examined in their homes by two visiting nurses. These subjects had received a letter in advance informing them of the arrival of the nurses

In the survey centres the B.P examination was the last event for each subject and care was taken to obtain standardized conditions and avoid disturbing factors, noise stress upon the subjects and staff etc The subject sat in a resting position at a table with the right upper arm at heart level Two sphygmomanometers were used on each person in strictly alternating sequence the conventional apparatus and the special apparatus of Rose et al (15) The two apparatuses had a main cuff 40 cm long and 14 cm wide Three pressures were read as follows the systolic at the appearance of the Korotkow sounds the diastolic 4th phase at the point of muffling of the sounds, and the diastolic 5th phase at the disappearance of the sounds The examinations were carried out by 19 registered nurses who had had a thorough training period in advance Detailed written instructions were given to each nurse and, in the first part of the survey their work was closely supervised During training, in the middle and at the end of the survey objective testing of the nurses was carried out by a tape recording of standard auscultation findings. The observer variation among the nurses of the study has been subjected to a detailed analysis (4)

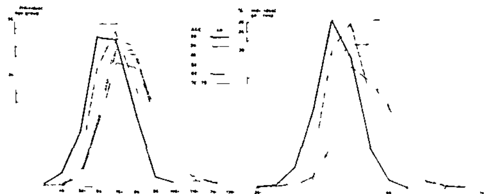


Fig 3 Diastolic 5th phase blood pressure in different age groups in males and females.

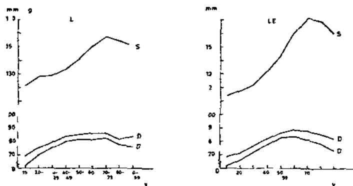


Fig 4 Mean blood pressures in age groups, males and females

RESULTS

The blood pressure findings using the conventional apparatus in the study are illustrated in Figs 1–7 while the detailed numerical values are to be found in the appendices

Fig 1 gives the frequency distribution of the systolic blood pressures found in the total random sample all ages and both sexes combined Skewing to the right side is apparent but no bimodal distribution is indicated

Figs 2 and 3 give the frequency distributions for each age group males and females separately Here in both sexes the modal value is displaced to the right in the older groups and at the same time greater dispersion and increased skewing are to be seen

Fig 4 gives the mean values of the three blood pressures for each sex in age groups The dispersion of the systolic blood pressure is indicated by the standard deviations in appendix *III* and by the values in appendices IV and V The range of pressures is seen to be widest in the oldest age group

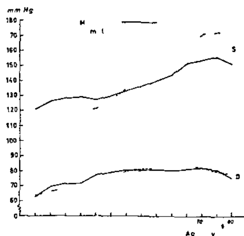


Fig 5 Median blood pressures in age groups males and females

Fig 5 illustrates the median value of systolic and diastolic phase 5 blood pressures in the two sexes Systolic values in females are seen to be lower than those in males up to the age of 45–49 years but in the higher age groups the conditions

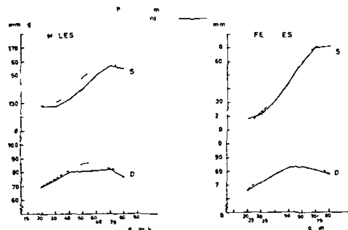


Fig 6 Mean blood pressures in age groups measured in private homes and in survey centres

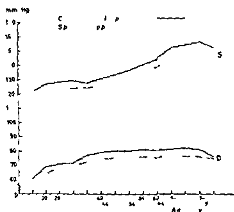


Fig 7 Mean blood pressures in males measured with conventional and with special sphygmomanometer

are reversed The same is indicated for the diastolic pressure but the difference between the sexes is smaller

In Fig 6 results from subjects examined in the survey centres are compared with those from subjects examined in their private homes Slightly higher values are found in the latter group This may reflect the different measuring conditions or real differences in the subjects or both

Fig 7 illustrates the differences between the results obtained by the use of the two types of sphygmomanometers The special apparatus is seen to give lower values than the conventional apparatus The details of this finding are discussed in another publication (4)

Appendix VII gives the correlation between the blood pressures and weight height and the coefficient W/H Here the systolic pressure has a correlation coefficient to weight varying between 0 140 and 0 267 in females and between 0 059 and 0 221 in males The coefficient in females varies with height from 0 006 to 0 377 and in males from 0 022 to 0 265 The correlation to W/H is no better

Thus the correlation between systolic blood pressure and height and weight may be said to be small The values are highest in the age group 40–69 years in females

In the two diastolic pressures the correlation with height and weight is of the same order as for systolic pressure The highest weight coefficients are found in the 30–49 age group in females −0 298 and 0 314 in diastolic 5th phase

The correlation between the systolic blood pressure and the pulse rate is of interest for the study of the influence of psychological factors upon blood pressure There is no great correlation in appendix X which suggests that variation in the forces affecting the pulse rate is not matched by variation in systolic blood pressure

The correlation between the systolic blood pressure and the two diastolic pressures has also been investigated and the results appear in appendix IX Here the correlation coefficient is higher than for any of the relationships mentioned above In the middle age groups the coefficient is about 0 6 and 0 7

DISCUSSION

The sample

In epidemiological studies the problems of representativeness of the group examined are repeatedly encountered Very frequently an ideal random sample is prepared in advance but it proves difficult to have the whole of this sample covered in the study (1 2 19) Even in small communities and in the cases of perfect vital statistical service practical problems are encountered in the fact that the population is undergoing changes (*a*) in the interval from the time point of the theoretical constitution of the sample until the actual survey is started and (*b*) during the survey In addition difficulties inherent in the term *residence* may arise These differ in various areas depending upon population structure conditions of work and so on

The Bergen Blood Pressure Survey of 1963 was based upon the population on July 1st The survey started on September 1 and was carried out during 4 ½ months of the autumn and early winter Thus only slightly more than 6 months passed from the point of constitution of the group to the end of the survey and consequently the dynamics of the population did not have too great an influence upon the sample However 56 subjects died within the survey period and 179 moved away Of the latter group 16 had moved out of the city without reporting to the statistical office in due time In these cases home visits by the two nurses brought the migration to light giving a corrective for the official register of the population as per July 1 1963

The problem of the 43 sailors who had been registered in Bergen but had no real residence

there may be met with in other communities having a similar sailor population The exclusion of these sailors from the sample hardly calls for strong objection

The percentage of the total sample actually examined was 96 2 This represented 98 1% of the subjects actually present in the city during the survey period Tables I and II show that the age group 20–29 years was unfavourably covered in the study and appendix II shows that in the youngest groups quite a number of the subjects were temporarily absent for such reasons as military service employment as sailors and studies and work abroad Some were in chronic hospitals penal institutions etc outside the city Consequently it was virtually impossible to get 100% attendance or examination coverage in a short survey period Similar experiences would be met in other city populations In the present study all possible means were used to examine the sample For example the visiting nurses paid as many as five visits to some subjects in their private homes before they were able to make the examination Those subjects of the sample who were patients in a mental hospital inside the city were all examined

In view of the way in which the sample was chosen in our study we feel satisfied with an attendance rate of 98 1% of the population present We know however that a number of the subjects listed as not examined have actually had their blood pressure measured for example the 52 men in military service These results have not been included in the analysis because the technical details of measurement have differed from those used in the rest of the study The 46 subjects examined in hospitals and by private physicians have not been included for the same reason However we conclude that the group examined in the present study is a representative sample of the Bergen population born before January 1 1950

The size of the sample has been chosen as approximately 10% of the total population This may be accep ed as a suffici ntly high proportion for sampling in general and for the purpose of the study in particular (6) The total of just over 8000 subjects allows for subgrouping according to age and sex and at the same time is not too great for detailed examination in a short period (The size of the sample was also kept low in this case because the same sample was planned to be used in a longitudinal study of cardiovascular conditions over many years and thus the dimensions had to be adapted to the capacity for such a follow up)

The blood pressure findings

Of the findings in the present study the mean blood pressure values demonstrate an increase from adolescence to the age of 75 years in both sexes which was most marked in females In males the mean systolic blood pressure was 128 mm Hg in the age group 20–29 years and 157 mm Hg between 70–79 years The greater part of the increase with age was seen between 40 and 59 years In females the mean systolic pressure was 118 mm Hg in the group 20–29 years an 171 mm between 70–79 years of age The increase with age was more gradual than in the males and was greatest between 50 and 59 years

With regard to diastolic pressures the sex differences were not so pronounced The increase from the young to the old age group mentioned above was from 66–82 mm Hg in females and from 69–82 mm in males for the fifth phase There too the intersection of the curves for the two sexes was found between 40 and 49 years

The blood pressure values in the present study are in agreement with several previous studies including the one in Bergen 1950/51 (1) The systolic values found in Group 2 in Bergen in 1951 are almost identical with the present findings in males as well as in females In Group 1 of 1950 the values were 5–10 mm Hg higher

The increase of blood pressure with age shows the same trend in this study as in several others The highest age groups 80 years and over show a more marked *decrease* than for example the findings of Master et al (12) and Edwards et al (3) but the relatively low values in this age were also found in Bergen 1950/51 (1) and in the New Zealand study of Veale et al (17)

As regards the absolute values Hamilton et al (5) and other reports from Britain show mean systolic pressures in males about 5 mm Hg lower than the present study while the findings in females agree very well The same holds for the reports of Master (11) and Comstock (2) from USA Karpinos findings in large numbers of young males in USA (9) tally quite well with

ours In Miall and Oldham s report (13) from a sample of the general population in Britain the results in males correspond well with ours while the findings in females are relatively higher in the older age groups

In the present study diastolic blood pressure has been measured corresponding to phase 4 as well as phase 5 Compared to the findings in Bergen 1950/51 the present phase 5 values are 5–10 mm Hg lower particularly in the older age groups Phase 4 values are more in accordance with the 1950/51 findings being 2–5 mm Hg lower in the young groups and about 5 mm lower in the old groups

Phase 4 seems to be used in several of the other population surveys and is thus preferred for comparison in the present study Here the results from USA reported by Master and by Comstock agree well with ours while the values found by Miall and Oldham in Britain are considerably higher especially in elderly females Otherwise the differences between the blood pressure in males and females vary little from study to study

Comparison of the results of blood pressure examinations in different areas and at different times can only be made with considerable reservations The casual blood pressure is influenced by so many factors and the variation in equipment reading technique and reader characteristics is so great that differences and similarities have to be judged with care However it is surprising that the absolute values and the age trend are so similar to those reported in different studies of various population groups even when the groups were chosen by very different methods It is interesting to note that the findings in the present representative sample of the Bergen population in 1963/64 are so similar to the results of the previous Bergen study in 1950/51 especially those from 1951 (1) In the first study the group examined was not a representative one but was very large comprising about 75 of the population

This point is further elucidated in the present study in the comparison between the findings in the majority of the subjects attending the survey centres and the rather small group of non attenders who were examined in their homes by the two visiting nurses The findings in the latter group may indicate the conditions of the individuals who are not included in ordinary studies such as the population survey of Bergen 1950/51 This might be a group of individuals with particular blood pressure and vascular conditions

The results in Fig 6 demonstrate that except in young females there are consistently higher blood pressure values in the group examined at home than in the group examined at the survey centres This difference may be a real one between the subjects examined or may be a result of variations in the details of the examination The two visiting nurses were chosen from the 19 nurses who had performed the main part of the survey and were thus highly experienced Their observer details were subjected to a thorough study but no particular qualities could be found concerning terminal digit preference variation between the two apparatuses used in each subject influence of fatigue in the daily work period influence of sex or age of the subject examined the height of the blood pressure measured and so on (4) The pulse rate was also examined for the two subgroups of the sample In females those examined at home showed slightly higher pulse rate than those examined at the survey centres In the males no such difference was present However the numbers examined at home were small

Thus the pulse rate does not indicate that the relatively high blood pressures in the males examined at home is associated with psychological factors Altogether it must be noted that the subjects not attending the survey centres demonstrate slightly higher blood pressures than those attending and that it is not possible to decide whether this difference is caused by the technical situation of the examination by particular qualities in the individual non attenders or by both factors Otherwise the correlation of systolic blood pressure with the simultaneous pulse rate in the same subject was found to be low This is not in full accordance with the indications from practical work with casual blood pressure when high blood pressure and high pulse rate may be found at the beginning of an examination and both decrease in repeated readings during a period of rest and relaxation There is no definite sex difference in this respect in the total material and the sex of the person carrying out the examination does not seem to carry decisive importance as shown elsewhere (4) These findings cannot

therefore support the vague indications given by Comstock (2) in this respect but the present study was *not planned to give a definite answer* to this problem

In this study no close connection was found between the blood pressure and the height and *weight in each subject (appendices VI and VII)* It must be remembered that the cuff used here was 40 cm long and 14 cm wide in order to reduce the influence of the arm circumferences The low correlations found here may be taken as a support of the findings of Karvonen (10) that the cuff size is important in this respect Thus it appears that the influence of the dif*ference in arm circumference and of the weight* interrelated as they are may be significantly reduced when a sufficiently long and wide cuff is used in the measurement

The absence of any relationship between blood pressure and weight was also found in the Bergen survey of 1950/51 (1) and in several other studies such as that of Master et al (11) Here *however the smaller standard cuff was used* It is of interest in this connection that Holland and Humerfelt (7) found no significant relation between (*a*) the differences in *intra-arterial* and standard *cuff pressures and (b) the arm circum*ference or the skinfold thickness

Finally the correlation found in this study between the systolic and diastolic pressure in the *same subject has been relatively high even higher* than that which was found in the large group of young males reported by Karpinos (9) This worker also indicated that the correlation increased with age *from 17 to 37 years but this is* not so pronounced in the present study

The Bergen Blood Pressure Survey of 1963 has tried to give a picture of the blood pressure conditions in a general population utilizing better methods of sampling and investigation technique than was used in the survey of 1950/51 The main results are however surprisingly similar The random sample of approximately 10 % of the Bergen population will be subject to a longitudinal study over many years and the findings of 1963 will be related to morbidity and mortality due to cardio vascular renal disease in the same group

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Appendix I *The random sample related to the total population of Bergen in age and sex groups*

	Males		Females		Both sexes	
Age (y)	Total pop	Sample	Total pop	Sample	Total pop	Sample
70>	3,577	334	6 185	558	9 762	892
0-69	5 458	519	7 732	761	13 190	1,280
-59	6 800	618	8 513	871	15 313	1 435
	7 464	689	8 156	764	15 6?0	1 453
9	6 419	599	6 711	667	13 130	1 ?66
9	7 356	608	8 506	725	15 86?	1 333
19	4 941	482	5 408	482	10 349	946
otal	42,015	3 849	51 211	4 774	93 6	8 6_3

Appendix II *Age and sex composition of the 329 subjects of the non attendance group of the random sample population of Bergen*

A *Tempo ary absence*

Age (y)	Abroad		Schools at sea etc		Institutions		Mil tary service	Total		Total Both sexes
	♂	♀	♂	♀	♂	♀	♂	♂	♀	
70-79					1	1		1	1	2
60-69	1	1	4	1	2			7	2	9
50-59			8	1				8	1	9
40-49	6		10	2	1		2	19	2	21
30-39	3	10	11		2	1		16	11	27
0-29	3	6	3	9			46	52	15	67
15 19	5	7	17	1	2		4	_8	8	36
Total	18	24	53	14	8	2	5_	131	40	171

B *Present but n t exami d*

Age y)	Examined hospital Private phys ian		Temporary difficulties		Conditions of work		Diseases		Bare refusals		Total		Total Both sexes
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	
		1						2				3	3
	1	2					2	3	1	2	4	7	11
79	1	4	1				1	2	5	8	8	14	22
0-69	5	8	2	6				1	2	7	9	2_	31
9	3	4	1		2		1		6	7	13	11	4
	6	5			3		2		7	5	18	10	_8
39		1	1	1	4				1	_	17	4	21
9	3	1	2	1	3	1			2	1	10	4	14
15-19	1		1	1					1		3	1	4
otal	20	6	19	9	1	1	6	8	5	3	8_	6	158

Appendix III *Numbers mean blood pressures and standard deviation in age groups*

Age (y)	*n*	$\bar{S}$	*s*	$\bar{D}_4$	*s*	$\bar{D}_5$	*s*
Females							
90–99	11	160 7	30 4	82 5	9 6	73 5	14 2
80–89	123	169 8	34 0	85 4	15 4	78 4	17 3
70–79	399	171 2	29 7	88 0	14 4	81 5	15 5
60–69	737	160 2	28 4	88 7	13 4	83 6	14 1
50–59	805	143 9	24 2	86 6	12 3	82 2	12 5
40–49	752	131 8	21 8	82 1	11 2	77 7	11 8
30–39	652	121 9	14 4	76 3	10 3	71 8	11 2
20–29	706	117 6	13 0	71 3	10 3	66 0	11 3
(15–19)	473	114 2	12 8	78 3	10 8	61 4	12 1
Total	4 658						
Males							
90–99	5	150 8	21 2	84 0	14 3	76 8	9 9
80–89	71	154 3	31 5	81 6	15 7	77 2	17 3
70–79	245	157 4	27 2	86 1	14 6	81 7	15 2
60–69	503	156 6	27 0	86 0	13 3	81 3	13 7
50–59	597	141 0	24 1	84 9	12 8	81 1	13 1
40–49	652	132 9	18 2	83 8	11 4	79 8	12 6
30–39	566	128 5	15 4	78 9	11 1	74 5	11 6
20–29	546	127 5	13 1	74 9	10 0	69 2	11 6
(15–19)	451	121 5	12 5	68 5	11 7	60 9	14 0
Total	3 636						

Appendix IV *Systolic blood pressure in age groups (females)*

mm Hg	15–19	20–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64	65–69	70–74	75–79	80–84	≥85
250–								1		3	3	3	1	2	
240–										3	4	2	3	2	
230–								1	3		5	6	2	1	1
220–							4	3	3	4	5	5	3	5	4
210–							2		6	4	8	11	13	4	2
200–						3	3	4	2	7	13	12	7	2	1
190–						1	4	6	10	24	17	18	12	4	1
180–					1	4	5	11	15	30	29	24	18	13	8
170–					5	8	15	15	32	43	50	31	31	10	3
160–		2	1	4	2	13	22	32	42	52	51	34	29	9	6
150–	2	5	7	7	9	15	29	42	57	61	53	24	21	14	5
140–	15	12	9	23	28	43	41	68	64	63	42	21	16	10	3
130–	37	46	39	47	54	77	74	84	73	61	25	14	14	6	4
120–	108	113	77	79	91	100	78	87	52	26	15	8	9	5	4
110–	158	127	101	96	102	70	64	41	25	21	11		3	1	
100–	105	73	50	48	37	37	31	13	12	3	1	3		2	1
90–	40	28	14	7	9	5	4	1					1	1	
80–	7	2		1	2										
70–	1														
Total	473	408	298	312	340	376	376	409	396	405	332	216	183	91	43

Appendix V *Systolic blood pressure in age groups (males)*

mm Hg	15 19	20–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64	65–69	70–74	75–79	80–84	≥85
60–											1	1			
250–								1							
[illegible]40–										1	1				1
230–								1	1		1	1			
2[illegible]0–								2	2	5	5			2	
210–								2	1	3	2	2	3	1	
00–						2		2	6	6	9	5	5	1	2
190–		1				1		5	7	4	9	12	7	3	
180–			1	2	1	3	8	7	11	13	14	12	5	4	3
170–	2	1		6	2	7	8	11	17	8	18	14	9	3	5
160–	2	5	3	3	6	14	21	16	28	29	29	16	12	1	3
150–	6	11	12	16	19	20	23	25	26	31	44	[illegible]5	12	5	2
140–	35	36	30	33	37	51	50	48	56	48	40	27	18	10	2
130–	66	72	62	81	63	67	83	61	66	48	35	18	7	9	5
1 0–	138	88	84	60	76	83	68	55	45	3[illegible]	24	10	6	5	2
110–	145	63	45	59	61	56	48	38	28	21	7	8	4	1	3
100–	48	21	9	22	14	22	10	13	13	9	5	4	1	2	
90–	9	1		1	4	4	3	1	2		1		1	1	
Total	451	[illegible]99	[illegible]47	283	283	330	322	288	309	258	245	155	90	48	28

Appendix VI *Diastolic—phase 4—blood pressure in age groups*

mm Hg	15–19	20–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64	65–69	70–74	75–79	80–84	≥85
Females															
140–								2	1	4					
130–								3	1	1		2			
120–							3	4	3	4	5	5	2	1	
110–				2	9	7	9	7	9	21	20	8	6	4	[illegible]
100–	1		2	4	4	9	25	29	41	41	40	27	24	11	2
90–	9	12	10	19	76	59	62	95	93	90	64	52	32	10	6
80–	59	60	50	75	89	101	128	134	109	1[illegible]2	111	49	43	15	5
70–	151	136	120	119	124	121	96	83	89	69	58	37	37	15	6
60–	138	93	65	59	44	32	22	9	9	16	8	9	8	7	1
50–	51	25	17	6	6	5		1	1		2		1		
40–	5	6		2	1				1						
30–	3	1													
20–	1	1													
10–		1													
Total	418	325	264	286	303	334	345	364	357	368	308	189	153	63	22
Males															
140–									1						
130–								1			1	3	1		
120–					1	2		2	7	4	5	1		1	
110–				1	4	5	11	8	5	4	10	6	1	1	1
100–	2	2	5	5	8	11	14	15	20	18	19	15	9	4	3
90–	7	11	11	26	32	57	65	56	48	46	49	27	17	6	5
80–	53	41	56	56	76	96	93	89	82	73	77	40	16	4	4
70–	121	84	61	96	66	74	73	64	67	55	40	34	21	13	3
60–	120	46	31	38	34	16	14	12	22	15	8	6	10	9	5
50–	48	11	5	1	1	2	1	1			2		1		
40–	6	2		1			1			1	1			1	
30–	3	1							1						
0–	2														
10–	1														
Total	363	198	169	2[illegible]4	[illegible]22	263	72	248	253	216	212	13[illegible]	76	39	21

Appendix VII *Diastolic—phase 5—blood pressure in age groups*

mm Hg	15–19	20–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64	65–69	70–74	75–79	80–84	≥85
Females															
140–										2					
130–								4		3		2			
120–							1	3	2	1	2	2			
110–				1		5	4	4	7	10	10	4	2	3	
100–			1	2	4	4	14	16	19	23	23	16	16	7	4
90–	2	3	6	8	17	32	46	63	60	71	50	32	26	8	3
80–	21	31	21	56	59	92	101	111	114	113	109	49	31	11	4
70–	84	94	82	93	119	118	127	122	107	96	78	48	46	15	5
60–	154	119	103	96	80	73	43	41	40	38	30	29	22	10	5
50–	103	63	39	18	22	14	6	2	3	9	6	3	7	7	
40–	28	16	7	7	1	2			2		1	1	1		
30–	13	4		2	1							2			1
20–	4	2	1	1		1							1	1	
10–	1	1													
Total	410	333	260	284	298	341	342	366	354	366	309	188	152	62	22
Males															
140–									1						
130–											1	2			
120–								2	6	1	3	2	1		
110–				1	2	5	5	7	3	2	7	3	1	3	1
100–		1	2	4	4	7	11	11	12	12	13	12	3	3	
90	4	4	4	14	23	33	35	32	37	31	39	22	14	2	5
80	22	16	29	35	49	76	85	75	71	68	61	35	19	5	5
70	63	60	56	72	63	89	92	85	68	68	59	37	20	11	3
60	108	67	55	77	48	45	37	31	47	26	19	19	14	8	4
50–	92	34	21	19	12	8	2	2	7	6	10	2	2	4	2
40–	45	11	2				3	1		1		1			
30–	15	2		1						1	1			1	
20–	5														
10–	4														
Total	358	197	169	273	201	263	270	246	252	216	213	135	75	37	0

Appendix VIII *The coefficient of correlation of* (A) *systolic and* (B) *diastolic 5 blood pressure to weight height and W/H² in age and sex groups*

	Females			Males		
Age (y)	Weight	Height	W/H²	Weight	Height	W/H²
(A)						
80–89	201	108	191	189	152	144
70–79	156	008	174	141	123	141
60–69	160	266	176	073	149	060
50–59	203	377	193	059	265	097
40–49	267	342	252	083	276	087
30–39	256	182	256	221	022	232
20–29	198	006	186	172	131	172
15–19	140	000	175	212	073	243
(B)						
80–89	192	051	168	071	117	081
70–79	158	036	170	216	114	200
60–69	158	047	176	155	149	175
50–59	242	082	230	175	090	218
40–49	314	186	331	207	030	217
30–39	298	107	311	240	052	253
20–29	161	015	169	167	131	180
15–19	051	080	103	197	157	218

Appendix IX *The coefficient of correlation of systolic blood pressure to diastolic 4 and diastolic 5 pressure in age and sex groups*

	Females		Males	
Age (y)	D 4	D 5	D 4	D 5
80–89	538	715	538	560
70–79	519	652	638	69?
60–69	611	685	612	716
50–59	649	717	680	7 3
40–49	676	749	618	07
30–39	589	681	595	643
0–29	403	489	376	435
15–19	329	466	258	340

Appendix X *Correlation of systolic and diastolic 5th phase blood pressure to pulse rate in the same individual*

Age (y)	*Males*			*Females*		
		Correlation coefficient			Correlation coefficient	
	n	Systolic	Diastolic	*n*	Systolic	Diastolic
(a) Total						
70–79	44	195	235	73	097	172
60–69	16[illegible]	244	274	249	097	063
50–59	317	247	261	455	178	07
40–49	361	351	30[illegible]	491	337	196
30–39	378	378	232	477	317	210
0– 9	319	383	290	414	375	2[illegible]6
15–19	309	257	080	434	420	208
(b) Measured at home						
70–79	8	– 210	– 544	24	201	121
60–69	16	– 016	203	36	– 057	139
50–59	23	385	387	21	– 046	– 302
40–49	37	348	397	37	583	054
30–39	36	231	188	[illegible]9	319	353
20–29	36	420	252	24	808	499
15 19	57	307	– 065	27	262	031
(c) M asu d in su vey centres						
70–79	36	268	355	49	026	288
60–69	146	269	281	213	117	228
50–59	294	243	249	434	185	052
40–49	324	350	289	454	302	217
30–39	342	391	239	448	316	198
[illegible]0– 9	283	372	93	390	339	214
15 19	252	247	103	407	430	225

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ON THE CONTENT OF MYOGLOBIN IN HUMAN MUSCLES

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Abstract A quantitative method of determining the content of myoglobin and hemoglobin in human muscle is described

A piece of muscle 1–4 g is ground with dry ice homogenized and extracted with 0.0[illegible] M phosphate buffer pH 7.4 After being centrifuged at 15 000 × *g* the solution is concentrated by dialysis against [illegible]5 poly ethylene glycol solution The hemoproteins are separated by gel filtration on Sephadex G 75 identified by the absorption spectra of their carbon monoxide compounds and quantitatively determined by the pyridine hemochrome method

The content of hemoprotein is calculated as per cent of dry weight, the latter being determined on the original homogenate

In an autopsy material mainly consisting of patients in upper age groups with chronic disease and modest physical activity average values of 0.9 g myoglobin per 100 g dry muscle were found in hearts (left ventricle) whereas corresponding values for diaphragm abdominal muscle and muscles of the thigh were 1.1 1.6 and [illegible] g per 100 g dry muscle It is assumed that values in healthy adults may well be somewhat higher From these values it is calculated that the total amount of myoglobin in an adult male is in the range of 1[illegible]0–150 g corresponding to an iron pool of 0.37 – 0.47 g or approximately [illegible] of the amount of hemoglobin iron

Comparisons with determinations arrived at by other methods are made and some aspects of the physiology of myoglobin and other hematin compounds are discussed

The insoluble or residual hematin has been determined It could be shown that the major part of this hematin can be accounted for by the mitochondrial hemoproteins the remaining part in all probability deriving from hemoglobin

In 1948 de Duve (11) presented a method of determination of myoglobin in human muscles which was based upon a simultaneous determination of myoglobin and hemoglobin in an extract of human muscle by means of spectrophotometry and a calculation of the total amount of hematin pigments as well as of the relative concentrations of each pigment Values from determinations of human myoglobin were given by de Duve (11) and Biorck (2) The latter found that skeletal muscle contained about twice as much myoglobin as heart muscle and that there was probably some relationship between myoglobin content and muscular activity However it was also found that a considerable amount of hematin remained in the muscle residue after homogenization and extraction It was not possible to elucidate the nature and the origin of the hematin and it was obvious that the relationship between the extracted and the unextracted hematin pigments might be of importance for the evaluation of the true content of myoglobin all the more so as the hematin content of the muscle residue was much higher in heart muscle where the myoglobin content was lower than in skeletal muscle where the myoglobin content was higher Attempts to investigate this problem further were made by Biorck and Nigro in 1956 (3) and it was clearly shown that the amount of residual hematin depended partly upon the solvent used in the extraction The extraction medium used by de Duve (11) and by Biorck (2) was acetate buffer of pH 4.5 and this was found to bring down the mitochondria to the muscle residue whereas for example saline kept the mitochondria in the solution It was also found in experiments with perfused and non perfused rabbit hearts that hemoglobin must contribute considerably to the residual hematin of heart muscle homogenates in the situation prevailing in the human heart viz where no perfusion has been performed

Since the publication of de Duve's and Biorck's studies remarkably few quantitative studies concerning human myoglobin have been reported in the literature (Table I) Drabkin (10) Perkoff

Table I *Content of human myoglobin expressed as g/100 g muscle dry weight*[a] *in various muscles*

Authors	Heart	Diaphragm	Abdominal		Extremity
Lehmann		2 0			1 0 2 25
Woodruff & Whipple 1926 (I died acutely II chronic disease)			I 3 2 II 2 6		I 3 9 II 2 6
Bywaters & Stead 1944					1 5–2 0
de Duve 1948	0 9–1 6		1 5–3 0		
Biorck 1949	1 5		2 0–3 0		2 0–3 0
Drabkin 1951				0 6	
Reynafarjé 1962			2 0		2 5
Perkoff 1965[b]				1 0	
Garby 1966[b]				0 4	
Åkeson et al 1968	0 9	1 1	1 6		2 2

[a] Values given as wet weight recalculated on the basis of dry weight ≈ 23 of wet weight
[b] No reports of material or methods given

(17) and Garby (12) have reported values considerably lower than those of de Duve and Biorck while Reynafarje (19 20) arrived at values for skeletal muscle similar to those of de Duve and Biorck (However only Drabkin and Reynafarje seem to have presented experimental data to support their calculations) The methods used by de Duve and by Biorck were elaborated at a time when ion exchange and gel filtration media were not yet available At that time there were only two ways to determine the myoglobin content of muscle either to make rather crude extracts with salting out procedures which invariably carried with them considerable losses of substance or to apply a spectrophotometric method applicable to a solution with more than one compound present as worked out by the above mentioned authors However in the following years chromatographic techniques rapidly developed and for some time we have been engaged on working out a chromatographic method for the simultaneous separation of myoglobin hemoglobin and cytochrome *c* from one and the same extract on one column We had also hoped that it would be possible to separate and quantitate the various myoglobins as has been described by Åkeson and Theorell for horse myoglobin (1) Our attempts failed but because of some interesting experiences an account of the experiments will be given

PRELIMINARY EXPERIMENTS

The material consisted of heart and skeletal muscle from routine hospital autopsies All operations were performed at cold room temperature

After the muscle was freed from fat and connective tissue it was cut into small pieces homogenized in an equal volume of water in a small scale MSE homogenizer for 1–2 minutes at 24 000 rpm and immediately neutralized with secondary sodium phosphate to pH 7 After being centrifuged for 45 minutes at 20 000 × g the residue was extracted once more and the two supernatants combined A third extract contained only negligible quantities of myoglobin and hemoglobin

The combined supernatants were oxidized with a slight excess of potassium ferricyanide dialyzed against 0 02 *M* phosphate buffer pH 6 4 and chromatographed on a column of carboxymethylcellulose equilibrated with the same buffer Three coloured fractions separated two of which could be eluted with the starting buffer while the third fraction remained at the top of the column. The first coloured fraction to emerge from the column was found to contain protoheme as evidenced by its pyridine hemochrome It showed hemoprotein—similar though unidentifiable spectral properties the most striking of which was a rather slow rate of reduction on addition of dithionite The second fraction contained only myoglobin while the fraction remaining at the top of the column was found to consist of hemoglobin and cytochrome *c* of which hemoglobin could be eluted with 0 02 *M* carbonate–bicarbonate buffer of pH 9 4 Cytochrome *c* was then eluted with 0 5 *M* ammonia

The unknown fraction which emerged first from the column presented the first obstacle to this otherwise promising method as it not only varied considerably in relation to total heme content but also accounted for a substantial part of the latter in some experiments on heart muscle more than ten per cent

We therefore focused our attention on this fraction and found that by chromatography on diethylaminoethylcellulose it could be resolved into five protoheme-containing fractions all associated with protein The largest of these five fractions was purified by electrophoresis and by spectral and amino acid analysis and ultracentrifugation could be identified as hemalbumin By incubation of human albumin Cohn s fraction V" at pH 8 and 37 C with a 2 fold molar excess of

Table II *Some clinical data concerning the subjects examined*

Autopsy serial no	Sex	Age	Duration of illness			Anemia			Myocardial fibrosis			
			Acute	< 1 month	1 month or more	None	Marked	Not known	None	Some	Marked	Not known
152 66	♀	81			+		+					+
167/66	♂	56		+			+				+	
177/66	♀	70			+	+					+	
187/66	♂	68	+					+				
188/66	♂	83			+	+						
189 66	♀	79		+		+				+		
199 66	♂	78			+	+						
23? 66	♂	6?	+					+			+	
236 66	♂	49	+			+				+		
253 66	♂	48			+		+			+		
?58/66	♂	69			+		+			+		
259/66	♀	49			+			+	+			
268/66	♂	69			+	+			+			
284 66	♀	59	+					+	+			
314 66	♂	81		+		+				+		
334 66	♂	78			+	+				+		
12 67	♂	57		+		+				+		
22/67	♀	45	+			+			+			
63/67	♂	51	+					+			+	
64 67	♀	80			+	+					+	
78 67	♀	71			+		+			+		

protohematin an apparently inhomogeneous methemalbumin could be formed This hemalbumin chromatographed on a carboxymethylcellulose column as a homogeneous fraction but separated on a diethylaminoethyl cellulose column into at least three distinct fractions one of which was identical with our purified hemalbumin fraction.

Further experiments showed that increasing dialysis time as well as oxidation with ferricyanide increased the amount of the hemalbumin-containing fraction All of this is consistent with a transfer of heme from methemoglobin which is known to have an appreciable dissociation at neutral pH (21) to other proteins capable of binding heme The fairly acid pH 6 4 during the dialysis favours the dissociation of hemoglobin as does the conversion by oxidation from a covalent to an ionic state This is less probable for myoglobin, which has been shown ( ) to have an appreciable dissociation at pH 6 6 in the reduced, deoxygenated but not in the oxidized state

As we have found the hemalbumin-containing fraction even in unoxidized undialyzed extracts it is obvious that it is at least partly preformed possibly postmortally in our material. The implications of these findings will be discussed later

As the chromatographic method outlined above gives adequate separation only at the particular pH used and when myoglobin and hemoglobin are present in the oxidized form this method is obviously unsuitable for quantitative work. *The possibility of determining all three of the hemoproteins then seemed out of reach and we decided to concentrate on the determination of myoglobin and possibly hemoglobin*

The problem dissolves into two parts first the complete extraction of all of the myoglobin and hemoglobin with as little destruction as possible and second the separation of these two from each other and from possible unspecific heme proteins

In the method to be described below the first has been achieved by exhaustive extraction in phosphate buffer at physiological pH and the second through separation of the components by gel filtration on a column of Sephadex G 75

THE NEW METHOD

Material

Determinations were performed on material derived from routine hospital autopsies This explains why the mean age of the subjects is so high which is, of course a certain disadvantage inasmuch as such persons have usually suffered from chronic disease with reduced muscular activity as indicated in Table II

Muscle tissue was excised from the apex of the left ventricle the diaphragm abdominal muscle and muscle of the upper thigh In most of the determinations 4 g of muscle were used for every single analysis The muscle tissue was stored at − 80 C if it could not be analyzed immediately

In most cases microscopical studies were performed on the muscle piece subjected to our study No gross pathology was found in muscular specimens other than the heart *The findings are reported in Table II*

Apparatus and chemicals

All centrifugations were performed in an MSE Super speed 50 S ultracentrifuge at a temperature of + 4 C A

Beckman DK 2 spectrophotometer with linear wavelength scale was used for recording spectra

The all glass homogenizer was of the Potter Elvehjem type with a total volume of 100 ml and a pestle diameter of 20 mm It was driven by a motor and the pestle speed was kept at 1500 rpm

Sephadex G 75 was obtained from Pharmacia Fine Chemicals AB Pharmacia Uppsala Sweden and the polyethylene glycol ?0 M from Kebo AB Stockholm Sweden

All other chemicals were of analytical grade

Methods

All the following operations were carried out at cold room temperature A piece of muscle approximately 4 g was dissected free of fat and connective tissue and dried carefully with filter paper The muscle was then cut into smaller pieces weighed and ground with dry ice in a porcelain mortar to facilitate the subsequent homogenization In the cases where duplicates were made a larger piece of muscle was first cut into smaller pieces and the mince then divided into two parts before grinding with dry ice

The mixture was then transferred to the homogenizer followed by 4 ml of a 0 02 M sodium phosphate buffer pH 7 4 and a few drops of dilute ammonia to bring the pH to about 7 4 The homogenization was then continued for a total of two minutes with two consecutive additions of 4 ml each of phosphate buffer The homogenate was then transferred to a tared centrifuge tube and the homogenizer rinsed with 4 portions of 4 ml each of phosphate buffer

The centrifuge tube was again weighed the homogenate well mixed and two aliquots of about 0 5 g transferred to tared weighing vessels and the accurate amounts noted The weighing vessels were then placed in a drying oven at 110 C and dried to constant weight usually over night After correction for the sodium phosphate of the buffer 2 42 mg per g of homogenate the dry weight of the latter corresponding to the actual amount of dry muscle present in the homogenate was calculated

The rest of the homogenate was then centrifuged at $15\,000 \times g$ for 45 minutes The sediment was rehomogenized with 16 ml of phosphate buffer and again centrifuged This washing process was repeated once again

The sediment and the combined supernatants were analyzed in the following way

The sediment and the combined supernatants were analyzed in the following way

and data from the work of Paul et al (15) Depending upon the consistence and amount of the sediment one fourth to one half was stirred up in three times its weight of 0 2 *M* sodium hydroxide containing 4 3 *M* pyridine After reduction with 5–10 mg of sodium dithionite the mixture was centrifuged for 20 minutes at $100\,000 \times g$ The light absorption of the pyridine hemochrome which is stable for at least one hour was recorded from 700 mμ to 480 mμ If the light absorption was very small a 2 cm cell was used as well as the expanded scale of the spectrophotometer

The difference between the absorbency at the absorption maximum between 554 mμ and 557 mμ the actual position depending upon the amount of cytochrome *c* present and at the minimum at 540 mμ was used for the calculation For practical reasons it was decided to calculate all primary data as μg of heme iron rather than as hematin or hemoprotein From the data of ref (15) it can be calculated that 1 μg of hematin iron per ml and per cm light path will give an absorbency of 0 445 Even if this method of calculation reduces the influence of background absorption due to opalescence and/or impurities it was sometimes necessary to make a correction for this absorption This correction was made by drawing a continuously increasing base line starting from the region 600 to 700 mμ in such a way that the two absorption minima of the pyridine hemochrome at respectively 540 and 503 mμ after subtraction of the base line show the same absorbency

The sediment from the centrifugation of the pyridine hemochrome was stirred up in a small amount of the same medium and checked with a hand spectroscope for the presence of hematin but never showed anything but traces of pyridine hemochrome bands

The combined supernatants were subjected to a separation on Sephadex G 75 on which myoglobin and hemoglobin can be separated from each other and from eventual unspecific heme proteins

The volume of the solution was at this stage about 55 ml and it was found that to get a satisfactory separation on a 2 × 55 cm column the applied volume should not exceed 4 ml Several methods for concentrating the solution were tried but the only method which gave full recovery was found to be dialysis against a strong polyethylene glycol solution To get a check on the total recovery of the concentration procedure a small aliquot was withdrawn before the concentration step and the total hematin iron content was determined as pyridine hemochrome as described above for the sediment

The remainder of the solution was then dialyzed against 400 ml of a 25 solution of polyethylene glycol ~0 *M* in 0 1 *M* sodium phosphate pH 7 4 containing 0 1 M sodium chloride

After about ?0 h dialysis the volume had decreased to ?–2 5 ml and the contents of the dialysis bag could be transferred quantitatively from the bag in a total volume not exceeding 4 ml A pyridine hemochrome determination was usually performed at this step to ensure complete transfer The solution was then applied to the top of a × 55 cl column of Sephadex G 75 previously equilibrated with 0 1 *M* sodium phosphate pH 7 4 with 0 1 *M* sodium chloride added to minimize tailing of the peaks The column was developed and eluted with the same buffer The flow rate was kept at 3 5 ml/h for the first 18h at which time hemoglobin and myoglobin had started to separate and was then increased to 15 ml/h 3 ml fractions were taken all the way and the light absorption of the fractions was measured at their respective absorption maxima in the Soret region i e 400–430 mμ

The absorbencies were plotted versus effluent volume as shown in Fig 1 Aliquots of all fractions showing light absorption in the Soret region were identified by

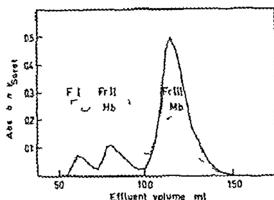


Fig 1 Separation of muscle extract on Sephadex G 75
— — skeletal muscle from autopsy no 12/67
heart muscle from autopsy no 259/66

the absorption spectra of their carbon monoxide-ferro compounds. The absorption maxima for the human myoglobin compound are 579 542 and 424 mμ while the maxima for the hemoglobin compound are found at 568 538 and 420 mμ. The first coloured peak to emerge from the column corresponded to the unspecific heme proteins described in the earlier part of this paper. Owing to a slight opalescence this peak was usually somewhat exaggerated as the measurements were made at rather short wavelengths

All fractions *containing myoglobin were then pooled* as were all fractions containing hemoglobin and the fractions containing the first peak which was called *fraction I*. In the overlapping fractions which were usually very small, a fairly accurate estimate of the relative concentrations of the neighbouring fractions could be obtained. A complete separation of the heme proteins could not be achieved without a considerable increase of the column size *which however was found* to decrease the recovery

Pyridine hemochrome determinations were then performed on all the combined fractions as well as on the overlapping fractions and the determined amounts of heme iron were recalculated to the original volume

The contents of hemoglobin and myoglobin could then be obtained by simply dividing the heme iron values by the respective *iron content, which for hemoglobin is* 0 347% (7) and for myoglobin 0 310%. The latter value is based on pyridine hemochrome and iron determinations on a sample of pure human myoglobin I prepared according to Akeson and Theorell (1) with a few modifications necessitated by the different starting material. This value agrees well with the molecular weight 17 900 reported by Perkoff et al (16)

RESULTS

The results from 43 determinations on 20 human subjects listed in Table II are presented in Table III

Comments

The values arrived at by the present method are somewhat lower than those reported by de Duve (11) Biorck (2) and Reynafarje (19 20) while at the same time the *relation* between values for skeletal muscle and heart muscle demonstrated by *Biorck (2) has been confirmed by our studies*

The reasons for the quantitative differences *pertain both to material* and method. As regards the *material* the muscle specimens in the present study derive from persons considerably older than those in Biorck's (2) previous material. This observation in a way reflects the changes in life expectancy mortality and composition of patient materials in Swedish hospitals over a period of 20 years. Thus the mean age of the patients in the latter material was 42 years for heart specimens and 51 for abdominal and skeletal muscle as against 60 and 66 respectively in the present material. As a matter of fact in Biorck's material the lowest values for skeletal muscle (below 2 per cent) were found in the oldest patients (50–75 years of age). Similarly the lowest values for heart *myoglobin (around 1 per cent) were found* in patients with cardiac hypertrophy and/or myo*cardial fibrosis. Similar autopsy findings* were observed in 11 of 15 cases in the present material. Therefore it would appear as if the mean values arrived at in this investigation are actually of the same order of magnitude as those given earlier by Biorck (2)

However there are differences between the two *methods* that per se might explain some differences in the results. Thus new constants have been worked out for pyridine hemochrome (15) and the iron content of myoglobin has been redetermined at 0 310%. The fundamental difference between the two methods however is that *de Duve's method represents* an estimation of two substances in a solution containing both whereas the present method gives a separation of each of the compounds in the extract. In general terms the former method is apt to result in higher values than the latter as separation procedures often are accompanied by some loss of substance. Furthermore our finding that unspecific hemoproteins e g *hemalbumin* are present *to a varying* extent in almost all extracts *from human* muscle derived from autopsies *invalidates the* assumption upon which de Duve's *method was* based viz. that the solution only contains hem-

Table III *Content of myoglobin hemoglobin fraction I and residual hematin in various muscles*

Determination on 4 g of muscle Brackets indicate duplicate analyses

Autopsy serial no	Myoglobin (g/100 g dry weight)[a]	Hemoglobin (g/100 g dry weight)	Fraction I (of soluble heme)	Recovery (of soluble heme)	Residual hematin (of total heme)
Heart					
152/66	1 44	1 83	2 37	100 3	5 87
167/66	1 41	0 74	8 65	78 0	7 71
167/66	1 00	1 31	3 11	86 1	11 53
177/66	1 10	2 72	1 26	92 9	5 57
189/66	0 35	2 16	7 18	93 1	14 05
232/66	0 95	1 64	8 84	102 2	—
232/66	0 80	1 47	8 12	98 3	—
236/66	0 81	2 97	2 30	90 9	8 98
236/66	0 70	2 66	7 57	96 4	8 79
253/66	0 91	0 95	5 21	105 5	6 38
253/66	0 92	0 88	2 22	102 3	9 79
259/66	1 17	2 92	4 73	108 1	4 07
284/66	0 91	1 83	4 66	93 7	10 05
314/66	0 74	1 87	1 50	93 9	24 44
334/66	1 08	2 14	2 44	96 2	8 35
22/67	0 67	1 46	3 72	82.6	20 50
63/67	0 89	1 64	1 12	91 2	5 20
78/67	1 15	1 27	3 35	95 4	5 83
	0 94±0 06	1 80±0 16	4 35±0 75	94 8±1 80	9 82±1 40
Diaphragm					
284/66	0 81	1 90	8 09	87 8	5 98
314/66	1 32	1 43	3 73	94 7	5 85
334/66	1 23	2 07	2 58	100 7	3 21
22/67	1 08	1 31	5 01	98 6	5 61
	1 11±0 11	1 69±0 17	4 85±1 19	95 5±2 83	5 16±0 65
Abdominal					
284/66	1 64	0 61	0 73	101 4	4 51
314/66	2 03	0 27	0 48	10? 5	3 50
334/66	1 20	0 60	2 20	93 6	4 43
22/67	1 41	0 35	0 74	95 9	4 23
	1 57±0 18	0 46±0 09	1 04±0 39	98 4±2 14	4 17±0 23
Skeletal					
152/66	3 01	0 59	—	101 9	1 59
167/66	2 64	0 43	—	99 7	1 69
187/66	? 78	0 62	—	99 2	2 07
188/66	1 33	0 51	3 93	99 2	2 85
199/66	1 60	? 14	2 81	97 5	4 28
258/66	2 40	0 35	1 64	93 6	1 86
259 66	3 02	0 13	0 85	100 4	2 54
268/66	2 48	0 60	0 91	106 9	4 57
284/66	2 39	0 26	1 08	100 1	2 05
314/66	1 82	0 51	2 55	104 7	2 74
334/66	1 78	0 59	3 07	87 1	2 55
12/67	2 ,0	0 47	0 77	101 9	3 02
22/67	1 66	0 67	1 49	96 1	3 56
22/67	1 16	0 44	0 96	72 6	—
63/67	2.15	0 39	0 44	95 5	0 63
78/67	2.64	0 78	—	102 8	0 48
78/67	2 82	0 63	—	102.2	0 35
	2 23±0 14	0 59±0 10	1 71±0 32	97 7±1 98	? 30±0 32

[a] Mean values given as mean ±s e of mean

globin and myoglobin We have tried to estimate the influence on the determination of myoglobin by de Duve s method of the presence of an average amount of hemalbumin in the extract and arrived at the conclusion that the myoglobin value becomes about 10 per cent too high in relation to the real content of myoglobin The effect is greater for heart than for skeletal muscle

d Duve chose acetate as extraction medium in order to produce as clear a solution as possible and by means of this the mitochondria were carried down into the residual hematin However sometimes the solutions became somewhat turbid and this turbidity although generally corrected for may also have increased the values

We also know that the extract contained cytochrome *c* If the wavelengths chosen by de Duve are used, the influence of cytochrome *c* is probably rather small but if the wavelengths used by Poel and others (18 19 20) are used this interference might be larger In the present method also some cytochrome *c* is released from the mitochondria during the extraction but this is eluted somewhat slower than myoglobin from the column and only negligible quantities can be found in the last myoglobin fractions

From these considerations it might be stated that the myoglobin value arrived at by de Duve s method are probably somewhat too high whereas the values arrived at with the chromatographic method described above are if anything somewhat too low At any rate the figures arrived at by the chromatographic method should represent minimum values. In view of the present knowledge it is rather remarkable that the values reported by de Duve (11) and by Biorck (2) agree so well with the present data.

As already mentioned the residual hematin" constituted a puzzling factor in Biorck s (2) earlier studies It is now clear that part of it was due to the fact that in de Duve s method the mitochondria were carried down into the sediment As the heart contains many more mitochondria per unit muscle than does skeletal muscle it is obvious that heart muscle residue must contain more hematin

As all the mitochondria are found in the residue after the extraction with phosphate buffer it is possible to get an estimate of the amount of "residual hematin that could originate from these Using Cleland and Slater s (8) value for the mitochondrial protein content of rat heart muscle 1 6% of the wet weight, which agrees with the total content of mitochondria in rabbit myocardium 12% of the dry weight (14) and usin Vanneste s (23) data for the cytochrome content of pure mitochondria one arrives at a figure of about 8 μg of heme iron in 4 g of wet muscle Even if only cytochrome *b* which comprises about 30% of the total heme iron in mitochondria contains protoheme all cytochromes will contribute in the pyridine hemochrome determination as they all have absorption maxima and minima at about the same wavelengths Cytochrome oxidase with the traditional absorption band at 587 mμ shows a gradual hypsochromic shift with time (13) with a maximum at 558 mμ and a minimum at 540 mμ which is very pronounced after 30 minutes the conditions of our determinations

As can be calculated from Table III 4 g of heart muscle contain an average amount of 8 μg of residual heme iron a considerably lower amount than that found by Biorck (2) and the same figure as found in the calculation of mitochondrial heme iron As is apparent from this work and from the work of Biorck and Nigro (3) part of the residual hematin can derive from hemoglobin and in all probability accounts for the residual hematin not attributable to mitochondria It has also been noticed in a few experiments that after extraction of the sediment with 0 4 *M* sodium chloride which releases only cytochrome *c* from the mitochondria the residual hematin determined as pyridine *proto*hemochrome decreased by about 25%

In the case of skeletal muscle the residual hematin iron only amounts to an average of 1 8 μg in 4 g of muscle which is a reasonable figure in view of the much lower content of mitochondria in skeletal muscle

In the beginning of our experiments with the chromatographic method we found it practical to work with muscle pieces of about 4 g which is more than the amounts used by de Duve and Biorck The chromatographic method however is more cumbersome and requires more time than de Duve s method which makes serial investigation of large materials difficult In order to make it applicable also to material from biopsi s or small laboratory animals the present method was subsequently tried also for 1 g samples The amount of buffer used in the extraction was in this case one fourth of that used for the 4 g samples otherwise the conditions were the same

As seen from Table IV the results for such determinations are about as reliable as those from 4 g samples

For reasons stated above we have therefore not attempted to make a new catalogue of the

Table III *Content of myoglobin hemoglobin fraction I and residual hematin in various muscles*

Determination on 4 g of muscle Brackets indicate duplicate analyses

Autopsy serial no	Myoglobin (g/100 g dry weight)[a]	Hemoglobin (g/100 g dry weight)[a]	Fraction I (% of soluble heme)	Recovery (of soluble heme)[a]	Residual hematin (of total heme)
Heart					
152/66	1 44	1 83	2 37	100 3	5 87
167/66	1 41	0 74	8 65	78 0	7 71
167/66	1 00	1 31	3 11	86 1	11 53
177/66	1 10	2 72	1 26	92 9	5 57
189/66	0 35	2 16	7 18	93 1	14 05
232/66	0 95	1 64	8 84	10⌞ 2	—
232/66	0 80	1 47	8 12	98 3	—
236/66	0 81	2 97	2 30	90 9	8 98
236/66	0 70	2 66	7 57	96 4	8 79
253/66	0 91	0 95	5 21	105 5	6 38
253/66	0 92	0 88	2 22	102 3	9 79
259/66	1 17	2 92	4 73	108 1	4 07
284/66	0 91	1 83	4 66	93 7	10 05
314/66	0 74	1 87	1 50	93 9	24 44
334/66	1 08	2 14	2 44	96 2	8 35
22/67	0 67	1 46	3 72	82 6	20 50
63/67	0 89	1 64	1 12	91 2	5 20
78/67	1 15	1 27	3 35	95 4	5 83
	0 94±0 06	1 80±0 16	4 35±0 75	94 8±1 80	9 82±1 40
Diaphragm					
284/66	0 81	1 90	8 09	87 8	5 98
314/66	1 32	1 43	3 73	94 7	5 85
334/66	1 23	2 07	2 58	100 7	3 21
22/67	1 08	1 31	5 01	98 6	5 61
	1 11±0 11	1 69±0 17	4 85±1 19	95 5±2 83	5 16±0 65
Abdominal					
284/66	1 64	0 61	0 73	101 4	4 51
314/66	2 03	0 27	0 48	102 5	3 50
334/66	1 20	0 60	2 20	93 6	4 43
2?/67	1 41	0 35	0 74	95 9	4 23
	1 57±0 18	0 46±0 09	1 04±0 39	98 4±2 14	4 17±0 23
Skeletal					
152/66	3 01	0 59	—	101 9	1 59
167/66	2 64	0 43	—	99 7	1 69
187/66	2 78	0 62	—	99 2	2 07
188/66	1 33	0 51	3 93	99 2	2 85
199/66	1 60	2 14	2 81	97 5	4 28
258/66	? 40	0 35	1 64	93 6	1 86
259/66	3 02	0 13	0 85	100 4	2 54
268/66	2 48	0 60	0 91	106 9	4 57
284 66	2 39	0 26	1 09	100 1	2 05
314/66	1 82	0 51	2 55	104 7	2 74
334/66	1 78	0 59	3 07	87 1	2 55
12/67	2 ⌞0	0 47	0 77	101 9	3 02
22/67	1 66	0 67	1 49	96 1	3 56
22/67	1 16	0 44	0 96	72 6	—
63/67	2 15	0 39	0 44	95 5	0 63
78/67	2 64	0 78	—	102 8	0 48
78/67	2.82	0 63	—	102 2	0 35
	2.23±0 14	0 59±0 10	1 71±0 32	97 7±1 98	2 30±0 32

[a] Mean values given as mean ±S E of mean

globin and myoglobin We have tried to estimate the influence on the determination of myoglobin by de Duve s method of the presence of an average amount of hemalbumin in the extract and arrived at the conclusion that the myoglobin value becomes about 10 per cent too high in relation to the real content of myoglobin The effect is greater for heart than for skeletal muscle

of myo_lobin in healthy adults is higher from all we know about the relation between activity and myoglobin levels Levels around 150 g would app ar more likely This is considerably more than the figure of 35 g once given by Drabkin (10) which still seems to be used in many cal culations

Because there has been some tendency in the lit rature (12) to underestimate the contribution of myoglobin to the iron pool of the body it should b stated that the figures given above indicate that myoglobin should contribute 3 1 mg iron per g of myoglobin or 400–500 mg iron per 70 kg (6–7 mg per kg body weight) The relation ship betwe n hemoglobin and myoglobin iron would be in the order of 5 1

In this investi_ation accordingly we pres nt a new method of estimating the myoglobin content of human muscle Its main advantage over the m thods used by de Duve (11) and Biorck (2) is that the myo_lobin is quantitatively collected from a column and analyzed separately from hemoglobin cytochrome *c* and unspecific hemoproteins The presence of the latter substances means that d Duve s method may involve a certain though moderate error in the determinations The values arrived at with the present method are generally somewhat lower than those previously reported by de Duve (11) and Biorck (2) How ever when account is taken of the differences in mean age of the subjects in the two materials the results are rather remarkably similar Our new values are probably lower than would be ex pected in healthy adults

The previously observed differences in myo globin content between heart and skeletal muscle have been confirmed In addition diaphragm seems to occupy an intermediate position while abdominal muscle has a lower content than muscles from the thi_h Such findings are in good a reement with the general concept of a relationship between the myoglobin content and the type of muscular activity In this respect there is an invers relationship between the con tents of myoglobin and cytochrome *c* as earlier reported by Biorck (4 5) whose findings on cytochrome *c* were recently confirmed by Dall man (9) This is per se an interesting fact not least in the phylogenetic perspectives of the differentiation of various hematin pigments for specialized purpos s and with regard to possible feedback mechanisms d termining the levels of these pigments This in turn must be considered in the light of the total hematin pool and the total iron pool of the body in which—as shown above —myoglobin plays a not unimportant role It would seem warranted to study in greater detail with the more specific methods now available the interrelationship and principles governing the concentration of hematin compounds in various tissues in clinical conditions where iron metabolism and/or tissue metabolism are interfered with

ACKNOWLEDGEMENTS

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Table I *Mean total blood volume plasma volume blood pressure exchangeable sodium serum sodium and body weight*

(*A*) befo e and after 1-2 weeks therapy with hydrochlorothiazide/KCl
(*B*) before and after 3 months therapy with hydrochlorothiazide/KCl

	Before	After	Change	S E M	*P*
(A)					
Total blood vol ml (10 pat)	5058	4878	−230 (4 6)	72	<0 0
Plasma vol ml (11 pat)	3109	2971	−138 (4 5)	40	<0 01
Blood pressure mm Hg (11 pat)	141	121	− 20 (14 2)	5	<0 005
Exchang sodium mEq (6 pat)	3293	3075	−218 (6 7)	64	<0 02
Serum sodium mEq/l (6 pat)	144 5	142 0	− 2 5 (1 7)	0 8	<0 05
Body weight kg (11 pat)	77 1	75 8	− 1 3 (1 7)	0 3	<0 005
(B)					
Total blood vol ml (9 pat)	5227	4921	−306 (5 8)	85	<0 01
Plasma vol ml (10 pat)	3170	2932	−238 (7 5)	60	<0 005
Blood pressure mm Hg (10 pat)	141	116	− 25 (17 7)	4	<0 001
Exchang sodium mEq (6 pat)	3293	3161	−132 (4 0)	26	<0 005
Serum sodium mEq/l (6 pat)	144 4	143 2	− 1 2 (0 9)	1 6	>0 40
Body weight kg (10 pat)	80 2	79 6	− 0 6 (0 9)	0 5	>0 0

were determined 1 to 2 weeks and a ain 3 months after the initiation of antipressor treatment and the values obtained were compared with those before treatment One patient died (stroke) before the 3 months were over and the determination of the erythrocyte volume was unsuccessful in another Exchangeable sodium was determined in six patients concurrently with the above mentioned studies

RESULTS

In the present study both plasma volume and total blood volume have been statistically analysed as the erythrocyte mass removed at the first determination is not able to reform by the time of the second determination 1 to 2 weeks later The fall in total blood volume is thus larger than the fall in plasma volume making total blood volume a better parameter than plasma volume Plasma volume has however been determined in most of the previous studies of the effect of thiazides on blood volume and has therefore been included here

Statistical data and average changes in total blood volume plasma volume blood pressure exchangeable sodium body weight and serum sodium are given in Table I It can be seen that there is a significant fall in average total blood volume of 230 ml (4 6 %) with short term treatment and of 306 ml (5 8 %) with prolonged *treatment The corresponding,* also significant falls in plasma volume are 138 ml (4 5 %) and 238 ml (7 5 %) respectively Mean blood pressure (systolic + diastolic)/2 fell by 20 mm Hg (14 %) and 25 mm Hg (18 %) Exchangeable sodium also fell significantly in both groups 218 mEq (7 %) in the short term group and 132 mEq (4 %) in the prolonged the serum sodium fall of 2 4 mEq/l (2 %) after 1 to 2 weeks of treatment was significant while the 1 2 mEq/l (1 %) fall after 3 months of treatment was not significant Th re was a significant fall in body weight of 1 3 kg (2 %) with short term treatment an insignificant fall of 0 6 kg (1 %) with prolonged treatment

Percentage changes in total blood volume plasma volume and blood pressure in individual patients are shown by Fig 1 After 1 to 2 weeks of treatment with hydrochlorothiazide there was a rise of 1 % in total blood volume in one patient while there was a fall of between 2 and 13 % in nine others Almost corresponding chances are seen in plasma volume with a small rise in on and a fall in the rest Mean blood pressure rose 12 % in one patient and fell 7 to 24 % in the others After 3 months of treatment there was a 4 % rise in total blood volume in one patient and a fall of 1 to 12 in the others (Fig 1) Plasma volume rose 1 % in one patient and fell between 2 and 18 % in nine Mean blood pressure fell 8 to 27 % in all patients in the prolonged treatment group

Fig 2 shows percentage changes in exchange

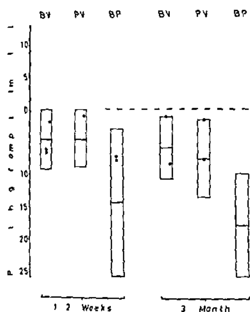


Fig. 1. The effect of hydrochlorothiazide on blood volume (B V) and plasma volume (P V) and on mean blood pressure (B P) (systolic + diastolic)/2 after 1 to 2 weeks and after 3 months of treatment. The solid line across each column represents the mean per cent change for the whole group. The upper and lower borders of each column indicate ± 1 S.D. respectively.

able sodium, serum sodium and body weight in the individual patients. There was a fall of 1 to 12 % in exchangeable sodium in all patients after

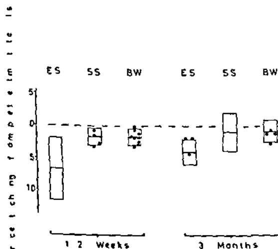


Fig. 2. The effect of hydrochlorothiazide on exchangeable sodium (E.S.), serum sodium (S.S.) and body weight (B.W.) after 1 to 2 weeks and after 3 months of treatment. The presentation of data is the same as in Fig. 1.

1 to 2 weeks of treatment. Serum sodium and body weight changed very little. After 3 months of treatment exchangeable sodium fell between 2 and 8 % in all patients while there were greater fluctuations in serum sodium and body weight than in the short term treated, serum sodium rising in two and falling in four and body weight rising in three and falling in six.

DISCUSSION

In the present study short term thiazide treatment produced a significant fall in total blood and plasma volume, exchangeable sodium, serum sodium and body weight. These results are essentially in agreement with previous studies. Thus Gifford et al. (10) and Conway and Lauwers (1) found a fall in plasma volume of 8.6 % and 10.7 % and in blood pressure of 9.1 % and 10.5 % respectively after one week of treatment. In contrast Gifford et al. (10) in spite of an increased natriuresis did not demonstrate a reduction in total exchangeable sodium, while the present study showed a significant decrease.

A significant fall in total blood and plasma volume and in blood pressure was seen in the patients treated for 3 months with hydrochlorothiazide. In addition there was a significant fall in exchangeable sodium, though smaller than that seen in the group treated for 1 to 2 weeks. On the other hand the fall in serum sodium and body weight was not significant. These results are essentially in agreement with those from Wilson and Freis (20) 1958 study. They found that plasma volume fell 8 % and extracellular fluid (thiocyanate space) diminished on an average by 2.5 l after 6.3 months of treatment. Serum sodium remained unchanged. In a later work, however, Wilson and Freis (21) demonstrated a significant fall in extracellular fluid alone, there being no significant fall in plasma volume after a treatment period of 6 months. After a year of treatment plasma volume as well as extracellular fluid had reached pretreatment levels even though blood pressure remained reduced. These same authors found, however, that if thiazide is withdrawn after several months of treatment, 5.4 on the average, plasma volume, extracellular fluid, body weight and blood pressure rise in the course of a week. Gifford et al. (10) and Conway and Lauwers (1) found that the plasma volume had

reached pretreatment level already after 1 month even though blood pressure remained reduced These same investigators reported no changes in total exchangeable sodium even after 2 to 8 1/ months of treatment They also observed that neither extracellular fluid (determined with Na) nor exchangeable sodium was influenced by 26 to 60 days on chlorothiazide (15) They did find however a significant reduction in body weight intracellular fluid and serum potassium It is not possible to explain why Gifford et al and Lauwers and Conway (10 15)—in contrast to the findings in the present study—found no fall in plasma volume and exchangeable sodium after prolonged treatment of hypertensive patients with thiazides even though they too showed that *thiazides have a sustained antihyp rtensive effect*

The mechanism by which thiazides lower blood pressure has not been completely elucidated Their initial effect can hardly be a direct anti pressor one as there is no fall in blood pressure in the first 24 hours after intravenous injection (3 4) With continuous administration blood volume and blood pressure fall in the course of a few days and as has been mentioned previ ously there is general agreement that this fall in blood pressure is the result of a reduction in blood volume and extracellular fluid one of the reasons being that an infusion of salt free dextran will imm-diately cause the blood pressure to rise (3 18 21) Further evidence is the observation that hypovolemia after short term treatment with chlorothiazide produces a fall in right atrium filling pressure and a fall in cardiac output (2 4 21)

The antipressor mechanism of prolonged treat m-nt of hypertension with thiazides is less clear Investigators who have not observed changes in blood and extracellular volume (1 15) have ad vanced the theory based on their own and others demonstration of a permanent fall in serum po tassium (15 21 22) and intracellular fluid (1) that the sustained antihypertensive action of thiazides is a result of changes in intra and extra cellular ion concentration producing dehydration of cells in the walls of the arterioles The blood pressure then falls as a consequence of a reduc tion in peripheral resistance due to this action on the arteriolar walls Conway et al (1) have shown that there is approximately a 24% fall in periph eral vascular resistance in hypertensives after 1–5 months of treatment with thiazides In ad dition it should be mentioned that thiazides may possibly reduce the sensitivity of the vascular walls to the normally present pressor substances Working with dogs Eckstein et al (5) were able to demonstrate a reduction in the vascular response to norepinephrine after both short term and prolonged administration of chlorothiazide

The mechanism of action of thiazides in lowering blood pressure is probably very com plicated especially with prolonged treatment The results obtained in the present study however suggest that a fall in blood volume and exchange able sodium in hypertensive patients treated both for 1–2 weeks and for 3 months with hydro-chlorothiazide may be a contributory factor in the reduction of blood pressure seen in these pa tients

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ALPHA METHYL DOPA (ALDOMET®) IN THE TREATMENT OF HYPERTENSION

The Effects on Blood Volume Exchangeable Sodium Body Weight and Blood Pressure

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Abstract Blood volume exchangeable sodium body weight and blood pressure were studied in ten hypertensive patients after 14 days of therapy with alpha methyl-dopa (Aldomet®) There was a significant increase in blood volume and a significant decrease in blood pressure Changes in exchangeable sodium and body weight were small and non significant One patient however developed cardiac pulmonary edema after one week of therapy

Following the demonstration by Sourkes in 1954 (22) that methyldopa inhibited decarboxylation a number of authors (5 6 14 20) have shown that synthesis of dopamine a precursor of norepinephrine from dopa is blocked in the presence of methyldopa On the basis of animal experiments it is known that decarboxylase inhibitors reduce both the catecholamine content of the brain heart and blood vessels and the serotonin content of the brain while at the same time causing a reduction in serotonin and dopamine excretion in the urine (3 10 20 22)

There are several reports on the hemodynamic effects of alpha methyl-dopa (AMD) Wincent (23) and Onesti et al (15) have shown that intravenous administration causes a fall in blood pressure in the recumbent position by reducing cardiac output and in the upright position by reducing both the cardiac output and the peripheral resistance Sannerstedt et al (19) have found that the reduction in blood pressure following long term oral intake is the result of a fall in peripheral vascular resistance Cardiac output on the other hand is not essentially changed and there is no effect on glomerular filtration and renal plasma flow

Weight increase the development of peripheral edema and of the symptoms of congestive heart failure have been described in hypertensive patients while under treatment with AMD (7 11 12) The increase in body weight does not seem to be progressive usually appearing only during the first few weeks of therapy Beck (2) found a significant increase in blood volume in several patients treated with AMD As there is only this one study of the influence of AMD on blood volume and no studies of its influence on exchangeable sodium the present investigation has been designed to study changes in these parameters in relation to the antipressor action of AMD

MATERIAL AND METHODS

Ten hypertensive patients were studied eight men and two women between the ages of 36 and 68 years None of the patients had clinical signs of congestive heart failure prior to treatment One of them had severely compromised renal function four had slightly reduced and five had normal renal function Advanced hypertensive retinopathy grade III–IV was found in nine one patient had normal eyegrounds Alpha methyl-dopa (Aldomet®) 250 mg two to four times daily was given to all subjects A standard diet containing approx 6 g sodium chloride daily was used Total blood volume was determined by a double isotope dilution technique with simultaneous measurement of erythrocyte volume using Cr plasma volume using ^{131}I albumin and exchangeable sodium using Na The technique used has been described previously by Hansen and Rønnow Jessen (8 9) Total blood volume exchangeable sodium body weight and blood pressure were determined 14 days after the beginning of Aldomet® therapy and the results obtained were compared with control values determined before therapy was begun The measurement of blood volume in case 3 was unsuccessful

Table I *Blood volume exchangeable sodium body weight and blood pressure in ten hypertensive patients before and after two weeks of treatment with l alpha methyl dopa (Aldomet®)*

Case no	Age	Sex	Total blood volume (ml) Before	After	Change	Exchangeable sodium (mEq) Before	After	Change	Body weight (kg) Before	After	Change	B P (mm Hg)[a] Before	After	Change
1	47	♂	4608	4873	+265 (+6)	3015	3127	+112 (+4)	71.8	72.0	+0.2	160/110 (135)	120/80 (100)	−40/−30 (−35)
2	68	♂	4615	4654	+39 (+1)	3251	3272	+21 (+0.6)	77.4	78.0	+0.6	210/115 (163)	185/110 (147)	−25/−5 (−15)
3	36	♂		–		3597	3392	−05 (−6)	86.3	87.0	+0.7	200/140 (170)	175/130 (153)	−25/−10 (−18)
4	41	♀	5703	6432	+729 (+13)	3673	3560	−116 (−3)	100.8	99.0	−1.8	190/150 (170)	160/120 (140)	−30/−30 (−30)
5	45		3931	4415	+584 (+12)	2468	2858	+390 (+16)	48.4	51.1	+2.7	210/120 (165)	170/100 (135)	−40/−20 (−30)
6	38	♂	4451	4745	−[illegible]06 (−5)	2738	2726	−12 (−0.5)	72.6	72.5	−0.1	180/1[illegible]0 (150)	135/105 (1[illegible]0)	−45/−15 (−30)
7	51	♂	4559	5085	+5[illegible]6 (+12)	3059	3150	+91 (+3)	67.7	69.7	+2.0	190/115 (153)	170/100 (135)	−20/−15 (−18)
8	58	♂	3635	4367	+732 (+20)	2822	2904	+82 (+3)	59.7	59.2	−0.5	195/105 (150)	200/100 (150)	+5/−5 (0.0)
9	55	♀	3003	3089	+86 (+2)	2085	2156	+71 (+3)	53.8	54.4	+0.6	205/115 (160)	150/110 (1[illegible]5)	−55/−15 (−35)
10	45	♀	4839	5262	+423 (+8)	2932	3130	+198 (+5)	69.2	70.2	+1.0	190/130 (160)	145/100 (123)	−45/−30 (−38)
Mean			437[illegible]	4714	+342 (+8)	2964	30[illegible]8	+64 (+2)	70.8	71.3	+0.5	193/122 (158)	161/104 (132)	−32/−18 (−25)
S.E.M					35			59			0.3			4
P					< 0.001			> 0.2			> 0.05			< 0.001

[a] The values in parentheses are mean blood pressure = systolic + diastolic/2

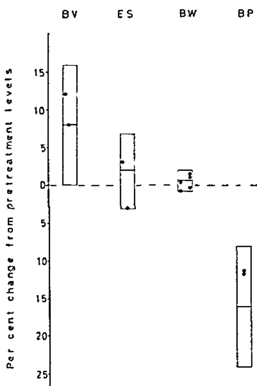


Fig 1 Per cent changes in blood volume (B V) exchangeable sodium (E.S) body weight (B W) and mean blood pressure (B P) (systolic + diastolic/2 after treatment for 14 days with Aldomet® shown by ●) The solid transverse line across each column represents the mean change for the whole group The upper and lower borders of each column indicate ±1 S D respectively

RESULTS

Values for the above mentioned parameters in each patient together with the mean values for the whole group are given in Table I There was a significant mean increase in total blood volume of 342 ml (+8) In only one patient did the blood volume decrease In the others there was an increase between 39 and 732 ml (+1 to +20) Changes in exchangeable sodium were rather modest and quite variable There was a decrease in three patients of 12 116 and 205 mEq respectively and an increase between 12 and 390 mEq in seven The mean change in exchangeable sodium was +64 mEq or +2 which is insignificant There was only minor fluctuation in body weight the mean change being an insignificant increase of 0 5 kg Three patients showed a decrease between 0 1 and 1 8 kg and seven an increase between 0 2 and 2 7 kg Body weight changes in patient no 8 require more detailed description There was a weight gain of 2 3 kg during the first seven days of Aldomet® therapy Signs of congestive heart failure appeared and there was an episode of cardiac asthma A single injection of mercaptomerin (Thiomerin®) was given and the patient was digitalized Body weight decreased 2 8 kg during the following week and the cardiac symptoms disappeared The total change in weight was thus a loss of 0 5 kg Mean blood pressure (systolic+diastolic)/2 for the whole group decreased by a significant 25 mm Hg in one patient (no 8) it remained unchanged in the others it decreased The per cent changes in blood volume exchangeable sodium body weight and blood pressure in each patient are given in Fig 1

DISCUSSION

In the present study an increase in total blood volume after two weeks of treatment with Aldomet® from 1 to 20 ′ was found in eight patients and a decrease of 5 was found in one Beck (2) studying blood volume changes in seven patients with weekly measurements during the first four weeks of therapy and with a single measurement after five months of therapy found somewhat different values There were considerably larger variations in blood volume after two weeks of therapy than found in the present study a decrease of 1 to 12 ₀ in three patients and an increase of 9 to 32 ′ in the rest After four weeks of therapy with Aldomet® Beck found changes between −8 and +75 in six patients and a decrease of 7 5 in one patient The changes at five months were much smaller The same investigator noted fluid retention in 11 of 15 patients two of whom developed peripheral edema weight changes are however not given She suggested that the increase in body weight which can be observed in patients treated with Aldomet® could be a result of an increase in blood volume Results obtained in the present study are difficult to compare with those obtained by Beck as different techniques were used to measure blood volume Beck employed a Volemetron apparatus and thus used a single isotope dilution technique (^{131}I albumin) A double isotope-dilution technique (^{1}Cr + ^{131}I albumin) was used in the present study in order to

correct for any changes in whole body hematocrit/large vessel hematocrit ratio that might arise during the study In the present study this ratio increased from 0 940 to 0 963 It is however questionable wheth r this fact alone can account for the much larger fluctuations in blood volume noted in Beck s study

An increase in body weight was seen in eight of ten patients The greatest increase was 2 7 kg and one patient developed congestive heart failure This is essentially what other investigators have reported Hamilton et al (7) recorded increases in weight up to 3 2 kg in 26 of 65 patients and noted the development of symptoms of congestive heart failure in three of them Bayliss et al (1) found weight increases up to 2 0 kg during the first week of treatment in six of 20 patients Finally Dollery et al (4) observed weight gains up to 6 kg in seven of 59 patients Four of these developed symptoms of congestive heart failure The above mentioned investigators state that body weight decreases as soon as Aldomet® therapy is discontinued or supplemented with a diuretic

Just like the changes in body weight the changes in exchangeable sodium were also non significant It seems reasonable to assume that these short term changes in body weight are the result of changes in body water and salt content The reason why there is no clear correlation between changes in weight and changes in exchangeable sodium (e g cases no 3 and 8) is probably because these changes are often smaller than the experimental error

There was a significant decrease in arterial blood pressure In only one patient (no 8) was there no change A completely satisfactory blood pressure (i e diastolic < 110 mm Hg) was obtained in six patients and a relatively satisfactory blood pressure (i e diastolic = 100–110 mm Hg) in two It was not possible in two of the patients to bring the diastolic blood pressure under 110 mm Hg A normal blood pressure level was reached however in only one patient but it must be remembered that nine of the ten patients studied had severe hypertension with grade III to IV hypertensive retinopathy These results agree well with those obtained by other investigators The consensus of opinion is that Aldomet® even after several months of administration produces a satisfactory decrease in blood pressure in only about one half to three fourths of hypertensive patients This holds true regardless of the type of hypertension—primary secondary malign or benign (12 13 19) It is stated however that tolerance to Aldomet® can develop (1 2 7 11) but that this can be compensated for by increasing the dose or by supplementing with a diuretic

CONCLUSION

Aldomet® is a very suitable antipressor drug even though it does produce an increase in blood volume as do also the sympatholytic and ganglionic blocking agents (17 18 21) Aldomet® however has two advantages 1 It lowers blood pressure both in the standing and in the recumbent position and produces only a slight orthostatic blood pressure fall (12 13 15) 2 It does not decrease glomerular filtration or renal plasma flow and it has only a moderate sodium retaining effect

The antipressor mode of action of Aldomet® is not completely understood but may in part be the result of norepinephrine depletion in the brain the heart or the peripheral vessels Thus in addition to an effect on the central nervous system there is also an effect on the peripheral sympathetic nervous system and the heart causing both a reduction in peripheral vascular resistance (19) and a fall in cardiac output (15) The antipressor action of Aldomet® is apparently not associated with catecholamine metabolism alone as the excretion of vanillmandelic acid a norepinephrine metabolite is not affected by Aldomet® (16)

ACKNOWLEDGEMENTS

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TRANSMISSION OF ATRIAL WAVES TO THE BLOOD VESSELS OF THE DIGITS AND OUTER EAR IN CASES OF ATRIAL FLUTTER

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Abstract 1 Digital plethysmograms pulse curves from the outer ear and from the brachial and radial arteries disclosed atrial waves in three cases of atrial flutter

The waves were sometimes more distinct in the plethysmogram from the finger than in the arterial pulse curve 3 Obstruction of the circulation by a cuff on the upper arm on several occasions did not affect the atrial waves in the finger while those in the pulse curve were wiped out 4 The waves disappeared on regularisation of the rhythm 5 It is concluded that the waves are transmitted not mechanically but in all probability nervously

Waves corresponding to the atrial contraction have been demonstrated in the arterial pulse by several observers (5) These atrial waves are easiest to study when the atrial and ventricular rhythms are dissociated as in A V block and atrial flutter

Are these waves transmitted to the periphery purely mechanically as is taken for granted by most observers or by a reflex mechanism as maintained recently? The latter conception was based on observations that contradict a mechanical propagation and on the fact that the waves could be eliminated by nerve block One of the observations was that the atrial waves showed little or no influence from friction and damping since they could be recorded in plethysmograms from the fingers A more extensive study of atrial waves in peripheral vascular sections has been undertaken to throw further light on their origin

METHODS AND MATERIAL

Digital plethysmographs of the piezo-electric type (Elema) described by Lund (6) were used The variations in volume of the tip of the digit which is enclosed in a cup with leakproof sealing are transmitted pneumatically to a manometer connected to a four-channel electrocardiograph (Elema Mingograph 4 B) Calibration is possible by introducing a constant volume of air (1 mm) into the system The arterial pulse was recorded by a piezo-electric microphone (4) placed on the brachial or radial artery The same type of microphone was used for recording the pulse of the lobule of the ear and was fastened by means of a springholder so that the pelotte of the microphone recorded the pulsatory variations in thickness

Three males with long standing atrial flutter (age range 46–56 years) were examined each of them on several occasions They were all ambulant with no signs of congestive heart failure or other diseases Two of them were digitalized In one of the cases the finger plethysmogram was followed during electrical reversion

RESULTS

Atrial waves of mono- or diphasic form and of the same duration as the flutter waves in the ECG were recorded in each case They were as a rule more conspicuous in the finger plethysmogram than in the peripheral pulse curve from the brachial or radial artery (Fig 1 *a*) They were sometimes also distinct in the pulse curve from the outer ear (Fig 2) and could be traced in the toe plethysmogram though less regularly and less distinctly than in the finger plethysmogram

In two cases obstruction of circulation by an inflated cuff on the upper arm did not affect the atrial waves in the fingers while those in the peripheral pulse curve were wiped out (Fig 1 *b*) in the third case the atrial waves in the finger were also wiped out

As seen in Fig 3 the atrial waves disappeared during electrical reversion already when the flutter at the first shock was transformed to auricular fibrillation

DISCUSSION

In this study of atrial waves in cases of atrial flutter it was found

1 that the atrial waves are transmitted not only to peripheral arteries but also to the minute blood vessels of the digits and outer ear

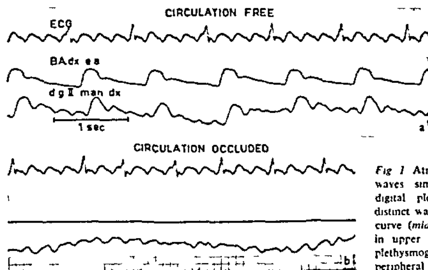


Fig 1 Atrial flutter in man aged 46 (*a*) Atrial waves similar to flutter waves in ECG in digital plethysmogram (*bottom curve*) less distinct waves in extra arterially recorded pulse curve (*middle curve*) (*b*) Circulation occluded in upper arm Atrial waves still distinct in plethysmogram while they are wiped out in peripheral pulse

2 that the atrial waves in the fingers are some times more distinct than those observed more centrally in the arterial system and
3 that the waves in the fingers are sometimes unaffected by obstruction of the circulation in the arm

These observations like those described in a previous work (5) are inconsistent with a mechanical transmission of the atrial waves They speak in favour of a nervous transmission—a reflex involving stretch receptors in the atrias and roots of the large veins with a quickly waxing and waning inhibitory effect on the sympathetic tone at each atrial contraction

The vascular sections investigated here disclose even small shifts in vasomotor tone (2) and are more sensitive to such shifts than peripheral arteries This may explain why the atrial waves are more distinct in the finger plethysmogram than in the peripheral pulse

Merrill and France (7) found that the atrial waves in the radial artery in a case of complete A V block disappeared when a cuff on the upper arm was inflated from this they concluded that the waves were not nervously transmitted in this case Blood vessels react differently however and we have seen that the atrial waves in the peripheral artery were eliminated by the occlusion while those in the finger were unaffected This observation has also been made repeatedly in normal subjects with sinus rhythm (Fig 4) The tension of the vascular wall may be of importance in this connection If it is lowered the tone of the smooth muscles is lowered according to Bayliss (1) This factor may be of greater importance for the arteries which have a higher wall tension than the small blood vessels of the fingers (Laplace s law) (3)

It may be concluded that the atrial waves are in all probability nervously transmitted They probably represent a key to our conception of vasomotor reflexes and show

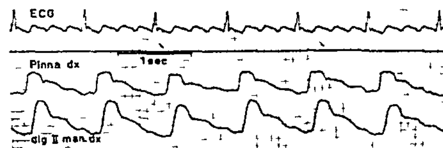


Fig Same case as in Fig 1 Atrial waves in digital plethysmogram (*bottom curve*) and in pulse curve from outer ear (*middle curve*)

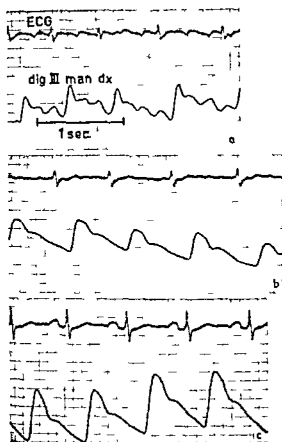


Fig 3 Atrial flutter in man aged 56 Electrical reversion ( ) Immediately before re ersion Atrial waves in finger plethysmogram. (*b*) Immediately after first shock when flutter was transformed to fibrillation Atrial waves disappeared (*c*) *Immed ately after second shock. Restora* tion to sinus rhythm No atrial waves

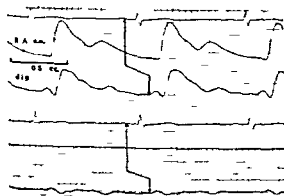


Fig 4 Healthy man w th sinus rhythm ( ) Distinct atrial wa es in finger plethysmogram (*botto cu e*) less d stinct atrial waves in extraarteriall recorded pulse curve from radial artery (*middle cu ve*) Tran m ss on time of atr al wave as measu ed from beginning of atrial systole (app ox mately 0 05 sec after beginning of P wa e in ECG) to upstroke of atrial wave in finger plethysmogram about 0 sec (*a row*) (*b*) Circulation occluded Atrial wa es still distinct in fing r plethysmogram while they are wiped out in pulse curve Transmission t me unchanged

(*a*) that the inertia of the effector system (the smooth muscles of the vascular wall) is less than hitherto considered

(*b*) that the reflex time is shorter than previously believed being of the same order as the transmission time of the pulse wave (Fig 4) (1)

(*c*) that there is a possibility of a coordination between the heart beat and the vascular tone

It may be proposed that by applying a brief str tch to the reflexogenic areas of the vascular system it should be possible to produce reflex responses similar to those produced by the atrial systole The results of such experiments will be described in coming articles

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EFFECT OF BRIEF CAROTID SINUS PRESSURE ON DIGITAL VASCULAR TONE

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Abstract 1 Brief carotid sinus pressure produced a rapidly occurring and passing volume variation of the digits as recorded by digital plethysmography in healthy subjects The first phase of the response was an increase in volume the second a decrease 2 The brachial arterial pressure was not affected by the pressure on the carotid sinus 3 The reaction was sometimes also elicited peripheral to an obstruction of the circulation in the arm 4 It is concluded that the response is probably due to a reflexly induced tonus variation of the vascular bed in the finger 5 The short latency of the reflex effect may denote a coordination between the heart beat and the peripheral vascular tone

It has been demonstrated that a brief pressure on the carotid sinus slows the atrial rhythm within a fraction of a second and that the effect waxes and wanes rapidly (8) Evidence has been produced to show that the characteristic arrhythmia in complete A V block consisting of postsystolic slowing of the atrial rhythm is due to a baroreceptor reflex induced by the ventricular systole (8) On these premises it was postulated that the peripheral vascular tone may also be reflexly influenced by the heart beat A probable sign of such an influence is the waves corresponding to the atrial contraction which are seen in the peripheral circulation when the atrial and ventricular rhythms are dissociated as in A V block and atrial flutter (12 17)

In the present work the theory of a reflex influence from the heart beat on the peripheral vascular tone was tested by applying a brief baroreceptor stimulus The effect was traced in plethysmograms from the digits which may disclose small shifts in peripheral vascular tone

METHODS AND MATERIAL

Digital plethysmographs (Elema) described in a previous paper were used (17) The manometers were mainly piezo-electric with a time constant of ? seconds In some cases a condenser manometer was used

The intra arterial pressure in the brachial artery was recorded by standard methods described elsewhere (3) In cases in which the intra arterial pressure pulse was not recorded the pulse from the brachial artery was registered extra arterially by means of a piezo-electric microphone (13) A standard pulse receptor (system Boucke–Brecht) was used for recording the pressure exerted on the carotid sinus

The subject rested in the recumbent position with the digits approximately at heart level Carotid sinus pressure was exerted bilaterally using the first and third finger of one hand and with the pulse receptor interposed so that the pelotte rested over one sinus Each pressure lasted about half a second The pressures which were made as alike one another as possible were exerted softly so as not to be disagreeable and not to produce vibrations of the body Several series of pressures with 10–15 second interval were performed The temperature of the room was 0–2 C The digits examined were fairly warm to the touch but maximal dilatation was not aimed at

Twenty-one healthy subjects (age range 11–40 years) were examined the majority of them several times

RESULTS

Carotid sinus pressure produced a rapidly occurring and waning volume variation of the digit in the majority of the subjects examined

The first phase was an increase in volume either a positive wave of the same duration as the applied pressure and at a constant interval (0 1–0 3 seconds) from the beginning of the latter (Fig 1) or an increase of the pulse wave immediately following the pressure (Figs 2 and 3) The second phase of the volume variation was a recoil to

carotid sinus press

P_{BA} dx — 1 sec.

dig IV man dx

b

c

d

Fig 1 Reaction to carotid sinus pressure in the digital plethysmogram (*bottom curve*) Distinct positive waves denoting volume increase of the digit. Interval from beginning of pressure to upstroke of produced volume reaction about 0.1 sec (*b c d*) Note suppression of next volume pulse in *c* and *d* Intra-arterially recorded pressure in brachial artery (P_B dx) shows no reaction to pressure on carotid sinus

decrease in pulse wave amplitude and a slight lowering of the pulse curve level (Figs 1 and 2)

The variation of the response was great both inter and intra individually

Quantitatively the maximal observed volume increase was of the same magnitude as the puls wave in the plethysmogram i.e. about 1–2 mm^3 (the volume of finger enclosed in the cup was about 3–10 cm^3)

The pressure curve from the brachial artery

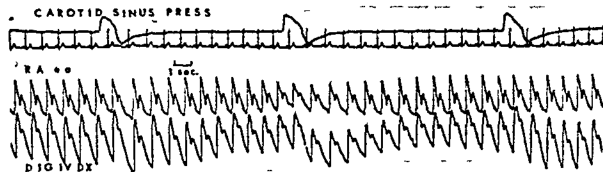


Fig Reaction of undulatory type of finger volume pulse (*bottom curve*) to carotid sinus pressure Extra arterially recorded pulse curve from radial artery (RA e.a.) is not affected

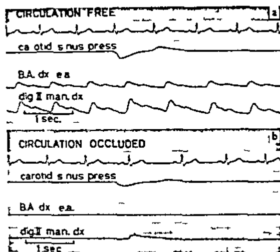


Fig 3 Reaction of finger volume pulse to carotid sinus pressure Slight increase of pulse wave following pressure (*bottom curve in a*) a similar increase of finger volume during occluded circulation

which was recorded simultaneously in the same arm as the digital plethysmogram in four cases showed no pressure wave produced by the impact on the carotid arteries and no significant pressure reduction from the brief baroreceptor stimulus The extra arterial pulse recorded in the other cases likewise showed no significant response (Figs 1–3)

When circulation was obstructed by a cuff on the upper arm no response to carotid sinus pressure was observed in the majority of cases but in some there was still a volume increase in the finger This occurred at the same interval as before obstruction (Fig 3)

This investigation was chiefly concerned with the reaction of the finger circulation Some curve from the toes were obtained as well however showing essentially the same responses to carotid sinus pressure as in the fingers

The impact on the neck sometimes produced small oscillations in the plethysmogram which were easily distinguished from the volume variations of the digits described above When a piece of string or adhesive plaster was wound tightly round the finger the volume reactions to carotid sinus pressure disappeared together with the volume pulse

COMMENT

A brief pressure on the carotid sinus produces a rapidly occurring and passing volume variation of the digit Since no concomitant variation of arterial pressure was recorded and since the reaction could sometimes also be elicited peripheral to an obstruction of the circulation of the arm the volume variations are not due to pressure variations Thus they must be caused by tonus variations of the finger vasculature In all probability these variations in tone are induced reflexly through the stimulus to the baroreceptors

Like the inhibiting effect on the S A node described previously (8) the effect is remarkably quick the latency being of the same order as the transmission time of the pulse wave from the aortic root to the finger and also of the same order as the transmission time of the atrial waves which as discussed elsewhere (12 17) in all probability are nervously transmitted Data on transmission velocities in autonomic nerves do not seem incompatible with the observed time course The crucial point is of course the velocity in postganglionic fibers Bishop and Heinbecker (4) have however shown that such fibers are not always non medullated and slow conducting Medullated fibers exist in some of the animals investigated Conduction rates around 5–18 m/sec have been described by Erlanger and Gasser (10) and 5–8 m/sec by Eccles (9) Little has been done experimentally however to assess the time course of baroreceptor reflexes The latent period for inhibition of the S A node is according to Brown and Eccles (7) and to Ashman and Gouaux (1) 0 10–0 17 seconds in dogs and according to Carlsten and Heyman (8) less than half a second in man Bayliss (2) found that blood pressure began to sink about 4–12 seconds after electric stimulation of the depressor nerve in rabbits but the latency for reduction of vascular tone is not assessable by recording the blood pressure The blood pressure reduction is a sluggish reaction being secondary to the reduction of tone in many different vascular areas What we observe here is the ripple of th effect "The large wave" of reduction of tone which produces the fall of pressure and masks the ripple is not brought into play by the brief stimulus Th se rapidly occurring and passing variations of digital volume produced by carotid sinus pressure are small and may seem to be of little functional importance But like the atrial waves which are probably produced by the stimulation of stretch receptors in the atrias and

roots of the large veins they signify that a co-ordination between the heart beat and peripheral vascular tone is possible It may be outlined as follows The systolic pressure rise by stimulating the sino aortic baroreceptors causes a reflex in hibition of the vasoconstrictor tone at about the same time as the arrival of the pressure pulse emitted by the ventricular systole The peripheral resistance is decerased for a fraction of a second and the outflow of blood is accordingly increased In this way the heart may steer the vasomotor tone pulsatorily Signs of a coordination of this kind have been described in previous articles (11–16) The cardiac grouping of discharge demon strated not only in the afferent fibers from the sinus and aortic nerves (5) but also in the efferent sympathetic fibers (6) may be the electrophysiological correlate to the described pulsatile influ ence on the effector organs Neil has proposed the same mechanism for the interaction between the heart and the peripheral resistance vessels as that described above (18)

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EFFECT OF RAPID DISTENSION OF LARGE ARTERIES AND VEINS ON THE VASCULAR TONE OF THE FINGERS

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Abstract 1 Finger plethysmograms were recorded in patients catheterized for diagnostic purposes 2 They disclosed a rapidly occurring and passing volume increase of the finger in connection with rapid flushing with saline or movements of the catheter against the vascular wall 3 Arterial pulse curves from the arm, recorded intra or extra arterially showed no effects on the blood pressure from the flushings or catheter manipulations. 4 The response was also obtained peripheral to an obstruction of the circulation in the arm. 5 The reaction is proposed to be due to a reflex produced by stimulating stretch receptors in the vascular system 6 The reaction was obtained not only when stimulating known reflexogenic areas but also *when stimulating other large central arteries* and veins.

Previous papers have reported that the atrial systolic waves are transmitted to the peripheral arteries (4) and the minut blood vessels in th digits and the outer ear (5) and it was conclud d that these waves are not tran mitted mechanically but reflexly It is possible to provoke such a r flex mechanism by a brief pressure on the carotid sinus (6) which produces a rapidly occurring and passing volume increase of the digits similar to that produced by the atrial systole

The aim of the present work was to imitate the presumed reflex effect of the atrial systole by brief intra vascular distension of reflexogenic areas such as the root of the aorta and the root of the vena cava inferior during catheterization for routine diagnostic radiographic purposes During the course of the investigation it b came apparent that similar effects on the vascular tone could be produced by the distension of other large central arteries and veins

METHODS

We used a standard digital plethysmograph of piezoelectric type a pulse microphone for recording the brachial or radial pulse and a standard pulse receptor for recording the pressure exerted on the syringe when flushing the catheter or the different movements made with the catheter In some cases the intra arterial pressure was recorded in the brachial artery These methods have been described in previous papers (5 6) Polyethylene catheters, no 05 with ? 4 sideholes about 3 cm from the tip were used The catheters were inserted percutaneously from the groin into the arteria or vena femoralis

As a rule the investigation was made after the radiographic examination. The catheter wa bri kly flushed with saline ?–10 ml sometimes it was rotated or pushed against the wall of the blood vessel In some case testing was done with several positions of the catheter during its withdrawal

MATERIAL

Twenty-six patients (age range 3?–68 years) were examined (Table I)

RESULTS

Flushing of the catheter produced a sudd n volume increase in the finger 0 1–0 3 second af t r the beginning of the injection The increase was of the same duration and shape as the pressure applied to the syringe The reaction mostly finished with a rebound to a volume d crease (Figs 1 and 2)

Movements of the catheter such as rotation pushing it against th wall of the blood vessel or pushing the leader of the catheter against the wall produced the same rapid volume increase

Table I. *Material*

Case	Sex	Age	Chief diagnosis	Roentgen examination
1	♂	59	Cysta ren. sin.	Nephroangiography
2	♀	57	Hypertens. Stenosis art. ren.	Venous arteriography
3	♂	66	Arteriosclerosis, hemiplegia	Venous arteriography
4	♀	50	Hyperthyreosis	Nephroangiography
5	♂	57	Arteriosclerosis, hypertension	Nephroangiography
6	♀	39	Albuminuria	Nephroangiography
7	♀	56	Nephrolithiasis sin.	Nephroangiography
8	♂	40	Hypertension	Nephroangiography
9	♂	51	Renal anomaly	Nephroangiography
10	♀	39	Pelvic varicosities	Pelvic venography
11	♂	47	Proctitis	Aortography
12	♂	64	Cancer pulm. op.	Aortography
13	♂	43	Cancer recti	Pelvic venography
14	♀	58	Hematuria	Nephroangiography
15	♀	68	Febris incerta causa	Aortography
16	♀	47	Hypercholesterolemia	Venous arteriography
17	♂	53	Stenos. ureter., hydronephrosis	Nephroangiography
18	♀	32	Observatio	Venous arteriography
19	♀	63	Cysta ren. dx., hypertension	Nephroangiography
20	♀	57	Hypertension	Venous arteriography
21	♀	40	Hematuria	Nephroangiography
22	♂	63	Status post. infect.	Venous arteriography
23	♂	40	Hypertension	Nephroangiography
24	♂	44	Hypertension	Aortography
25	♂	50	Tumor ren. dx.	Nephroangiography
26		40	Renal anomaly	Nephroangiography

in the cases that reacted to the flushing of the catheter (Fig. 1 c).

The brachial pulse curve recorded intra- or extra-arterially showed no pressure response to these manoeuvres and there was no concomitant influence on the sinus rhythm.

Obstruction of the circulation in the arm by inflation of a cuff on the upper arm did not

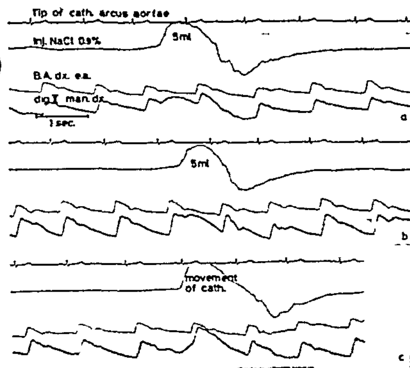


Fig. 1. Case 6. Effect of flushing of catheter tip in arcus aortae. Volume increase of finger with rebound to decrease (dig. II man. dx), no response in extra-arterially recorded pulse curve from brachial artery (B.A. dx. e.a.). Movement of catheter produces same effect (c).

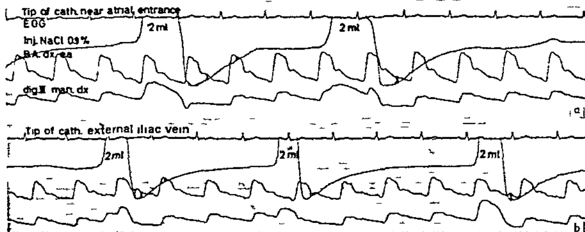


Fig 2 Case 2 (*a*) Effect of flushing of catheter tip near atrial entrance Volume increase of finger with rebound to decrease (dig III man dx) No response in extra arterially recorded pulse curve from brachial artery (B A dx e a) (*b*) Same effect with tip of catheter in external iliac vein

Table II *Results*

Position of catheter (tip)	No of subjects tested	No of cases with pos response
Aortic root	1	1
Aortic arch	7	4
Abdominal aorta	12	2
Art iliaca	6	3
Art renalis	3	0
Vena cava inf (root)	7	5
Vena cava inf (abdominal)	6	5
Vena iliaca	7	3

interfere with the response which occurred at the same interval as mentioned above (Fig 3)

The response was obtained in 13 out of 26 tested subjects (Table II)

COMMENT

We have observed a rapidly occurring and passing volume increase of the fingers in connection with brisk flushings and movements of catheters introduced in large arteries and veins

The patients did not feel the manoeuvres made with the catheter—on some occasions they even fell asleep during the investigation No movement artifacts were observed

Effects on blood pressure and heart rate have been reported in connection with intravascular injections of contrast m dia (1 9 10) No such effects were observed here The blood pressure and heart rate did not change The response was also obtained peripheral to an occlusion of the circulation This excludes passive distension of the finger vasculature by a pressure wave The latter fact and the rapid occurrence of the volume variation exclude the remote theoretical possibility of local influence of the injected saline on the vascular bed in the finger

The only remaining explanation seems to be that the slight local stretch of the vascular wall by the flushings and movements of the catheter reflexly influences the tone of the finger vasculature The prerequisite for such a reflex mechanism is the existence of stretch receptors As a matter of fact responses were also produced by stimulating wellknown reflexogenic areas (7) such as the root and arcus of the aorta and the root of the vena cava inferior at the entrance to the right atrium The large number of responses obtained from the latter area (Table II) imitate the effect of the atrial systole as predicted and confirms that the atrial waves (4 5) are reflexly transmitted

Identical reactions were however elicited from other vascular areas than the known reflexogenic ones (Table II) Stretch receptors of Pacini type have been described in and around the adventitia of large arteries and veins (8 11 12) They are very sensitive (3) Bursts of spikes synchronous with the systolic pressure rise have been

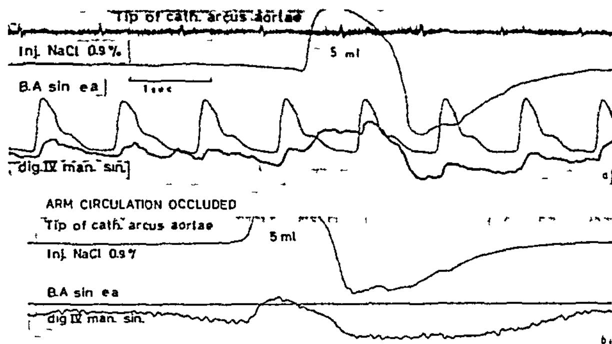


Fig 3 Case 4 Effect of flushing of catheter tip in arcus aortae *Volume increase of finger during free circulation (a)* and similar effect during occluded circulation *(b)*

recorded in nerve twigs coming from such corpuscles (2) but the reflex effects of such an afferent discharge are unknown

We cannot rely on the present investigation to *map out the position of such receptors in the* vascular system since the conditions associated with the studies were far from ideal we could often not pursue the studies as planned the environment was noisy and cramped the patients suffered from various diseases, they were often nervous and their digits cold Any of these factors may have influenced the results

It may be concluded that there are signs of a rapid reflex interference with the peripheral vascular tone through stretch stimulation of reflexogenic areas both the wellknown ones and those less acknowledged in large arteries and veins The possible function of such a reflex mechanism may be a coordination of the heart beat and the peripheral vascular tone as described previously (6)

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EFFECT OF MOVEMENT AND EXTERNAL PRESSURE ON THE VASCULAR TONE OF THE FINGERS

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Abst act 1 Finger plethysmograms were recorded during pass ve and acti e movements and pressure in vola manus and planta pedis and on the muscles of the upper and lower leg 2 They showed a rapidly occurring and passing volume change of the digit 3 Some movements were characterized by an initial increase in volume some by a decrease On cea ing the movement or releasing the pressure there was a recoil to the opposite reaction 4 Spontaneous variations of the responses were observed and different fingers sometimes reacted d fferently 5 The reaction was also obtained peripheral to an obstruction of the circulation 6 It is proposed that the observed effects are due to vasomotor reflexes produced by the stimulation of stretch receptors in the locomotion system

A previous article (12) reported a rapid volume change of the fingers in conjunction with the distension of large arteries and veins This response was elicited not only from reflexogenic areas such as the first part of the aorta and the root of the inferior vena cava but also from other large central blood vessels It was suggested that the reactions obtained from the latter areas are due to stretch receptors in and around the adventitia (20) Such receptors are also common in the locomotion system (8) With this in mind we looked for similar responses in the finger plethysmogram to movements and to external pressures especially in vola manus since this part is richly supplied with stretch receptors (4 19)

METHODS

The methods ha e been des r bed in previous art cles (9 10 11 1) In add tion to finger plethysmography we used a standard pulse receptor for recording the movements and pressures performed and somet mes also for recording the resp ratory movements The intra arterial pressu e in the brach al artery was reg stered in some ca es (1)

PROCEDURE AND MATERIAL

The reactions to the following movements were examined passive and active extension and flexion of the contralateral wrist passive extension of the fingers (one by one) passive extension and flexion of one knee passive and active dorsi and plantarflexion of one foot and passive extension of the big toe The reaction to pressure in the contralateral vola manus was extensively examined and on several occasions in addition the response to pressure in planta pedis and on various muscles such as the flexors of the lower leg and the extensors of the thigh

The passive movements were performed by fixing the extremity proximal to the jo nt with one hand while the other hand moved the finger the hand the lower leg or the foot to full extension or flexion kept it there for a short while and then relaxed the pressure For the active movements the subject pressed the hand or foot against the counterpressure of the invest gator s hand The movements were recorded by interposing a pulse receptor as described elsewhere

The pressure in vola manus was applied by pressing the subject s hand aga nst the pulse receptor which was interposed between the supporting table and the hand The pressure was thus concentrated to the pelotte of the receptor (diameter 12 mm) which was mostly placed between the thenar and hypothenar The pressure on the muscles was achieved by squeezing

The manoeu res were as a rule made w th the subject supine the arms resting comfortably on supporting tables. The e aminat on room was peaceful Room temperature was about ? C The examination was begun after the subject had rested for about 15 minutes

Forty healthy subjects (13–50 years of age) were examined many of them on several occasions.

RESULTS

In the majority of the subjects the finger plethysmogram displayed a characteristic reaction in the form of a rapid volume change i e an increase or decrease occurring 0 1–0 4 second after the



Fig 1 Reaction in plethysmogram from dig IV man dx to passive dorsiflexion of dig. III man sin. Upper tracing free circulation lower tracing circulation obstructed by cuff on upper arm Downward pointing arrow denotes beginning of pressure on finger upward pointing arrow denotes release of pressure on finger Note volume increase of finger shortly after beginning of pressure and recoil to volume decrease when pressure is released

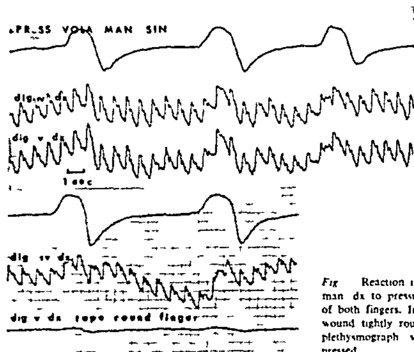


Fig Reaction in plethysmograms from dig IV and V man dx to pressure in vola man sin Volume increase of both fingers. In lower tracing adhesive tape has been wound tightly round part of dig V inserted in cup of plethysmograph volume reaction and pulse waves suppressed

beginning of the manoeuvre and followed by a recoil to the opposite reaction when the movement or pressure ceased (Figs 1–3)

As a rule the response was equally distinct when the circulation in the arm was occluded by a cuff on the upper arm (Fig 1) Thus it was mostly easier to analyse the response during occluded circulation without disturbing pulse waves.

The reactions to passive and active movements of the foot were analysed most extensively sin

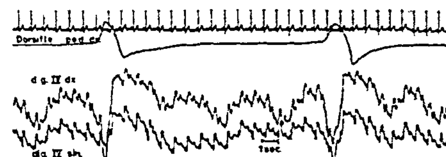


Fig 3 Reaction in plethysmograms from dig IV man dx and sin to passive dorsiflexion of ped dx Volume decrease followed by recoil to increase when pressure on foot is released

they were the liveliest and most consistent of those tested Passive dorsiflexion produced an initial volume decrease while passive plantar flexion produced an increase Active dorsi and plantarflexion produced responses opposite to the passive ones (Fig 4) Similarly passive extension of the knee elicited an initial decrease in volume while passive flexion produced an increase Movements of the hands and fingers passive as well as active as a rule produced an initial volume increase (Fig 1) and so did pressure in vola manus (Fig 2) and planta pedis Responses were also obtained though less consistent and weaker when squeezing the flexor muscles of the lower leg and the extensor muscles of the thigh

The variations in finger volume were not related to any concomitant blood pressure variations or to the respiratory movements of the chest The variability of the response was considerable both inter and intra individually Dif

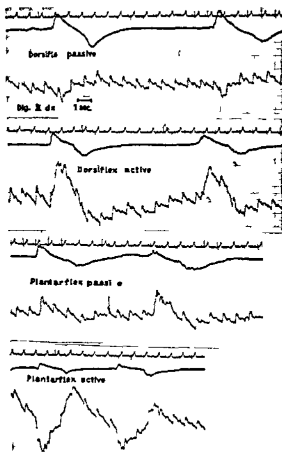


Fig 4 Reaction in plethysmogram from dig II dx to passive dorsiflexion passive plantarflexion active dorsi flexion and active plantarflexion of ped dx Volume decrease to passive dorsiflexion volume increase to passive plantarflexion while active movements produce opposite reactions to passive ones

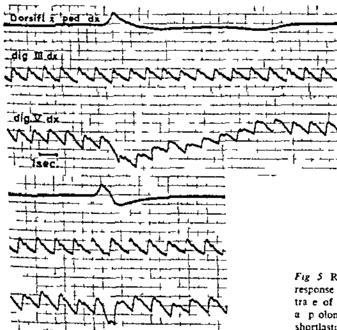


Fig 5 Reaction to passive dorsiflexion of ped dx Lively response in plethysmogram from dig. V man. dx only trace of response in dig III dx. Note also difference of a prolonged pressure on foot (*upper tracing*) and a shortlasting pressure (*lower tracing*)

ferent fingers sometimes reacted differently (Fig 5)

Movement artifacts were investigated by sup pressing the volume variations of the part of the finger which was inserted in the cup of the plethysmograph by winding a piece of string or adhesive plaster tightly round it The reactions described above then disappeared together with the volume pulse (Fig 2) They were thus due to volume variations and not to oscillations of the finger caused by movement Such disturbances were easily avoided with the technique used for the movements and pressure in question

COMMENT

The rapid variations observed in the volume of the finger are due to a sudden change of the relation between inflow and outflow of blood in the part of the finger enclosed in the cup of the plethysmograph this is not caused by a change in arterial pressure since the reaction was ob tained with the circulation occluded Thus it must be caused by a sudden change probably reflexly induced in the tone of the finger s vascular bed similar in type to that produced by the atrial systole (10) pressure on the carotid sinus (11) and distension of large arteries and veins (12)

There is an extensive literature on vasomotor reactions in fingers to various unspecific external stimuli (2 3 16) such as noise light touch pain etc but they are always vasoconstrictory and have a latent period of several seconds thus they do not seem to have anything to do with the present reaction

Circulatory reactions to stimulation of specific somatic afferents have been described in animals (14) Of special interest in this connection is the fall in pressure and increase in blood flow in various vascular beds produced by mechanical stimulation of muscles (13 17) Unfortunately it is difficult to compare these and other similar experiments on anaesthetized often decerebrate or spinal animals with experiments of this kind on nonanaesthetized man

The receptors are evidently widely spread in the locomotion system as well as in the cardio vascular system In the experiments involving vola manus firm pressure was needed to elicit any response touch alone did not suffice Pain was avoided but if produced it elicited a vasoconstriction with a slow waxing and waning influence on pulse wave amplitude and a latency of several seconds Since touch and pain do not produce the characteristic rapid volume reaction in the finger this must be due to some other type of receptor Stretch receptors for instance Pacinian corpuscles (18) may fit into the picture but only direct stimulation of such receptors is likely to give a definite answer

The effectors are the smooth muscles of the finger vasculature though it is of course unknown where in the complicated vascular architecture the reaction occurs The arterio venous anasto moses (6) seem to be of special interest in this connection on account of their lively rapid stopcock activity (7) their short latency and the rich supply of such structures in the fin er pads (6)

The state of the effector system is evidently of importance for the response This is demonstrated by the following observations (*a*) The reaction to one and the same stimulus may vary consider ably during the course of the investigation (*b*) Different fingers may react differently (Fig 5) and this variation is paralleled by different reac tivity to other vasomotor activities—the respira tory undulations the alpha deflections and the atrial waves (*c*) The response may be wiped out during reactive vasodilation after the finger has be n made ischemic by winding a string round it (Fig 2) During such a vasodilation the other signs of vasomotor activity mentioned under (*b*) also disappeared (as has been shown previously by Rein (15))

As we have seen some movements tend to produce an initial volume increase of the di it while others produce a decrease The results from passive and active movements of the foot suggest that this may have something to do with stretch of antagonistic muscles The opposite reaction to passive extension and flexion of the knee also tallies with this scheme but the response to the hand and finger movements does not fit since both extension and flexion produ e a volume increase of the contralateral digit

The recoil to the opposite reaction on relaxa tion of the pressure applied (for instance when extending one finger or in vola manus) is seen only when the release is sudden If the pressure is maintained or released slowly the initial response passes in a few seconds without any

such recoil (Fig 1) We have observed the same recoil in experiments with pressure on the carotid sinus (11) and flushing of large central arteries and veins (12)

The effects of the manoeuvres on the finger volume are small being of the same magnitude as the volume pulse i e about 0 5–4 mm³ for an enclosed finger volume of 3–10 ml They are however isolated effects of one movement It s ems possible that the aggregate effect of large muscle groups working rhythmically may influ ence the tone to a greater extent and thus also affect the flow It should be noted that Christen sen et al (5) found a practically instantaneous decrease of blood flow in the finger at the start of work on a bicycle ergometer This effect was attributed either to cortical influences or to reflex influences from the working muscles

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FAMILIAL OCCURRENCE OF M COMPONENTS

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Abstract Two families with multiple occurrence of M components are reported In one family a mother and her son had M-components The latter had myeloma with γGK and γAK M-components, the mother had a probable myeloma with γGK M-component Investigation of eight close relatives revealed no further M-component In another family three siblings had M-components A sister had a myeloma with exclusively light chain (Bence Jones) myeloma protein while two brothers had asymptomatic γGK M-components and Bence Jones protein uria respectively (the Bence Jones proteins in both cases were of type lambda) The significance of genetic factors in these familial cases is discussed in relation to the known figures of the frequency of M components and myeloma in Swedish populations

For some years we have had under observation two families with M-components occurring in two and three members respectively The occurrence of familial M-components has been regarded as rare but has been increasingly considered in the literature during the last few years It has connections with some questions of current interest as genetics of dysproteinaemia in general prevalence of M-components and "premyeloma Therefore we find it justified to present this report

Familial occurrence of M-components has been reported in at least 3 families.

Familial occurrence of myeloma has been described in 1 families (1 5 7–10 14 16–18 3 24) commonly in two siblings, occasionally in three siblings (1) or aunt and niece (3)

Myeloma and asymptomatic M-component occurring in the same family has been reported in couples of siblings in three families (13 23) (The asymptomatic M-components belonged to the types γG (13 23) and γM (3)) The clue to the findings of Spengler et al (3) was a systematic investigation of 110 relatives of individuals with M-components of different kinds (myeloma 14 macroglobulinaemia Waldenstrom six and asymptomatic M-component two)

In a similar study of 65 patients with *macroglobulinaemia Waldenstrom* 16 close relatives were investigated and in seven families a further eight individuals with γM M-component were found with or without clinical signs of macroglobulinaemia ( )

Two couples of siblings in two families had macroglobulinaemia in one of these families the mother had asymptomatic γM M-component Two couples of siblings in two other families had macroglobulinaemia and asymptomatic γM M-component respectively In the three remaining families the son had macroglobulinaemia and the mother asymptomatic γM M-component. (In an earlier paper two unpublished families were mentioned (1 p 50))

In this study no persons with γA or γG M-component were found but such components may occur in relatives of patients with macroglobulinaemia (2 p 145)

In another report (3) macroglobulinaemia occurred in a man and asymptomatic γM M-component in his daughter

Familial occurrence of asymptomatic M-components has recently been described in a population study from Sweden (2) Among 6995 individuals more than 25 years of age M-components were found in 64 Seven of these occurred in three families No cases of myeloma or macroglobulinaemia were found in these families.

Three and two siblings respectively in two families had G and γA M-component in a third family a woman had γG and a cousin of her father M M-component

Investigations of further relatives of the 64 individuals revealed no more cases with M-components

In another study of relatives of 1 persons with G or γM M-components without clinical signs of myeloma or macroglobulinaemia further cases with essentially asymptomatic M-components were disclosed in three families (6)

Two siblings had M M-component mother and son both had G M-component two siblings had A M component a third sibling had both γA and G M component and a niece A M-component

MATERIAL

Family I

Since it came to our knowledge that the mother of a man with myeloma known by us had been treated for the same disease during at least 1 year several relatives have been investigated clinically and electrophoretically Paper and immuno-electrophoretic investigations were performed at the Central Laboratory of Clinical Chemistry General Hospital Malmo (Head C B Laurell M D

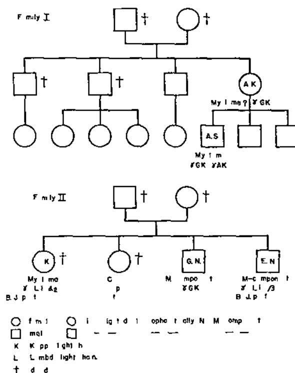


Fig 1 Pedigrees of the patients with familial M components

In cases of proteinuria with positive Heller test urine has also been investigated electrophoretically No further cases with M components have been found See pedigree Fig 1

Case I

A S a man born in 1915 developed symptoms of back pain and tiredness in 1964 In February 1965 he had anaemia (Hb 11 0 g%) increased ESR (58 mm/h) compression of a thoracic vertebra and osteolytic lesions in the femora Paper electrophoresis showed 5 1 g of γ globulin Immuno-electrophoresis revealed two M-components γAK and γGK (K = kappa light chain) Somewhat polymorphic plasma cells were found in the bone marrow (7 5)

Case

A K born in 1885 mother of A S described above developed increasing anaemia from 1950 In 1954 Hb was 7 4 g and ESR 115 mm/h maximally Paper electrophoresis total protein 9 2 g 43 of which was γ globulin The skeleton showed a general osteoporosis Bone marrow abundant occurrence of reticular cells partially in clusters some of them with two to three nuclei Since 1954 she has been treated some 20 times in hospital every time with blood transfusions In June 1965 compression of two lumbal vertebrae was found but no osteolytic changes Since 1957 a M-component of type γGK has remained constant at a concentration of 3 g that is during almost ten years The bone marrow smears have also been essentially stationary from 1954 to 1966 The last time in October 1966 18% plasma cells were found polymorph in appearance multinuclear and in clusters and special techniques (staining of iron sideroblasts etc) revealed changes consistent with haemolysis (Central Laboratory of Clinical Chemistry Sahlgren s Hospital Goteborg) Other signs of haemolysis have been observed at least since 1961 moderate reticulocytosis (commonly about 20‰ maximally 54‰) increase of bilirubin (4 0 mg maximally) haptoglobin below 25 mg Hepatosplenomegaly has been observed since 1954

Family II

A woman died in myeloma with a M component exclusively of low molecular type (Bence Jones type γuL, detectable in both serum and urine electrophoresis) Two brothers are alive without clinical signs of myeloma but both have M components γGK and Bence Jones proteinuria type γuL respectively A sister died in cancer corporis uteri some years ago Her serum was not investigated electrophoretically See pedigree Fig 1

Case I

S K a woman born in 1897 with back pain for several years became worse in December 1963 Osteoporosis and compression of thoracic vertebrae was found as well as a transient hypercalcaemia Paper electrophoresis showed increase of α (1 2 g‰) and slight decrease of albumin Hb was 11 0 g and ESR 45 mm/h Serum creatinine was 0 5 mg In August 1964 the pain increased and extensive osteolytic lesions and more compressed vertebrae were found Bone marrow typical picture of myeloma with 24 of plasma cells Paper electrophoresis showed atypical α configuration and by immuno-electrophoresis a γuL (L = lambda light chain) M component was found in α_2 This component was found al o by electrophoresis of the urine (Bence Jones protein 5 3 promille) Hb was 9 3 g% and ESR 77 mm/h Serum creatinine was 3 1 mg% and serum calcium 7 mEq/l After a clinical deterioration she received melphalan treatment from November 1964 and improved considerably both clinically and biochemically serum creatinine decreased to 1 5 mg% serum calcium was normalized and the concentration of the M-component decreased in serum and urine

In spite of this the skeleton lesions became more extensive Soon a rapid general deterioration occurred and she died The diagnosis of myeloma was verified by autopsy

Case

G N born in 1903 brother of S K was essentially healthy before 1963 when he was hospitalized for an uncomplicated cardiac infarction He also developed stiffness of the humeroscapular joints Occasionally an intrathoracic goitre was found In October 1964 protein-bound iodine was increased (11 0-17 5 μg) but there were very slight clinical signs of thyrotoxicosis Paper electrophoresis showed γ-globulin (1 9 g) with a M component Immuno-electrophoresis in February 1965

showed a M component of type γGK (0 9 g) in the slow γ region. Roentgenological examination of the skeleton showed normal findings. Bone marrow picture 3 plasma cells without signs of myeloma Hb 1? 8 g% ESR 4 mm h The results of paper and immuno-electrophoresis bone marrow and roentgenological exam ination of the skeleton were unchanged in October 1966 Hb was 10 8 g% and ESR 18 mm/h During the following few months ESR increased to maximally 58 mm h and the electrophoresis showed evident signs of activity with increase of the α fractions The cause of these findings could not be made sufficiently clear but the patient developed more distinct signs of thyrotoxicosis clinically and biochemically In January 1967 the M component and the bone marrow picture were un changed Hb 10 8 g ESR varied between 21 and 84 mm/h

Cas 3

E. N born in 1907 brother of S K has been essen tially healthy except for a moderate shoulder stiffness in May 1966 which improved rapidly Haemoglobin was 1_9 g% ESR 17 mm/h and serum creatinine 1 ? mg Albuminuria was found Paper electrophoresis revealed no M-component but immuno-electrophoresis showed a *quite atypical distr bution of light chain L suggesting the* occurrence of a minor M-component of type γuL in the β_2-region In the urine an abnormal excretion of Bence Jones protein type L was found At a check in April 1967 he was clinically healthy Hb was 1_5 g and ESR 14 mm h. Serum creatinine had increased to 1 8 mg% Renewed paper and immuno-electrophoresis on serum and urine showed the same picture as in May 1966 (the urine contained 1 4‰ protein ? 1 g in ?4 h) Roentgenolog cal exam nation showed no lesions indi cating myeloma Bone marrow picture in April 1967 3 5 plasma cells with sl ght s gns of immaturity

DISCUSSION

The finding of M-components in families can be adequately evaluated only in relation to studies of their occurrence in the population In adults the frequency of M-components is about 1 (3 4) and in geriatric series about 3% in people above 70 years of age (11 12) In a study of myeloma about four new cases occurred in 100 000 individuals of the population per year It was indicated that 12 myeloma patients were alive in 100 000 individuals of the population (26)

These figures indicate that two M-components may occur in a family by chance In our family II in which three siblings were affected one with myeloma and two with M-components with out clinical symptoms of myeloma (in one ex clusively in the urine) hereditary factors may be of significance Since population studies on th occurrence of M-components in the urine are lacking we cannot adequately estimate the role of such factors It has been suggested that a possible genetic background may be a disturbance of the control of the synthesis of the immune globulins (6 22) rather than a single gene ab normality with regard to the occurrence of dif ferent types of M-components in the same family Furthermore an increased frequency of other disturbances of immune globulins was found in the relatives of patients with macroglobulinaemia (22) Changes of immune globulins in relatives have been observed in other disorders with disturbances of the immune globulins and familial occurrence polyclonal hypergammaglobulinaemia for example was a common finding in primary acquired hypogammaglobulinaemia (27) and systemic lupus erythematodes (15) Of special in terest is the finding of both polyclonal and monoclonal hypergammaglobulinaemia in the same family The mother of a patient with pri mary acquired *hypogammaglobulinaemia had an* asymptomatic γG M-component two siblings and three paternal uncles had polyclonal hypergamma globulinaemia and another paternal uncle died of myeloma (27)

Case 3 in our family II is of special interest The abnormal production of Bence Jones protein in this practically healthy man was not detectable by paper electrophoresis He was investigated because we knew that his sister had suffered from myeloma and his brother had an asymptomatic M component The nature of his proteinuria had not been detected by the routine investigations of a health control *The abnormal excretion of* Bence Jones protein and the increasing serum creatinine suggests that a malignant condition will develop (cf 12)

A follow up of cases with asymptomatic M components of familial occurrence seems im portant particularly if myeloma or macroglob ulinaemia is known in the family as in our family II By following our cases continuously and by extending the investigation to other members of the families we hope to be able to throw some more light on the relationship be tween asymptomatic benign M-components and M-components of evident clinical significance (cf 12 19 20 22 25)

Addendum Since this manuscript was submitted another paper by Seligmann et al (cf 21 22) on

immunoglobulin abnormalities in families of patients with macroglobulinaemia Waldenstrom has been published Seligmann M Danon F Mihaesco C & Fudenberg H H Amer J Med 43 66 1967

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PROGNOSIS FOR JUVENILE DIABETICS WITH LATE DIABETIC MANIFESTATIONS[1]

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Abstract Prognostic studies were made on a series of 101 juvenile diabetics (onset before the age of 30 years) who developed late diabetic manifestations in the form of retinopathy and/or proteinuria between 1944 and 19 8 and in whom the beginning of these manifestations could be dated with reasonable accuracy Retinopathy developed after the diabetes had been present for 11–?0 years average 16 9 years the nephropathy as a rule some years later The patients were followed for an average of 9 5 years after the first compl cation had been diagnosed About half the patients developed persisting proteinuria during the follow up period Of this group 63 ,, died 83 developed uraemia and 58% severe retinopathy as a rule accompanied by permanent visual impairment The other half did not develop pers st ng proteinuria in spite of a longer duration of d abetes Only 2 of this group died 6% developed uraemia and 25 severe retinopathy Factors of prognost c mportance appear to be 1 Appearance of proteinuria before or within five years after the d agnos s of retinopathy – Occurrence of pers st ng prote nuria 3 Proteinuria exceeding 0 – 4 Occu rence of retinopathy and or nephropathy before the diabetes has been p esent for 15 years 5 Repeated ep sodes of diab tic coma 6 Onset of diabetes between the ages of 10 and 0 years

It is well known that after having been present for a number of years diabetes mellitus is nearly always complicated by generalized angiopathy (3) In juvenile diabetics this angiopathy manifests itself mainly as retinopathy which may lead to blindness and as nephropathy which may lead to death (1) The longer the duration of th- diabetes the more common the occurrence of retinopathy and nephropathy Thus 15–20 years after the onset of diab tes about 60 of juvenile diabetics (onset before the age of 30) have retinopathy and about 25 nephropathy

The patho enesis of diabetic angiopathy is still unelucidated It is also unknown why some diabetics fare better than others True the quality of the diabetes control appears to play a decisive role the risk of developing proliferative retinop athy and/or uraemia being less in patients whose diabetes has been under good control (5) How ever other factors must be of at least equally great importance

Not much is known about how juvenile dia betics fare once they have shown the first signs of retinopathy or nephropathy and it is uncertain whether it is possible to indicate at an early stage which patients are going to fare worst

In an effort to answer some of these questions juvenile diabetics who developed retinopathy and/ or proteinuria between 1944 and 1958 and in whom the onset of these late diabetic manifesta tions could be dated with reasonable accuracy were followed

MATERIAL

The material comprises 106 juvenile diabetics All were followed for five years or longer after the retinopathy and or nephropathy had been diagnosed Two patients were excluded because the course was compl cated by typical glomerulonephrit s 3 and 13 years respectively after the onset of diabetes Anothe two patients were excluded one because she d ed n a t affic acc dent fou yea s after the f rst sign of retinopathy and the other —a deaf and-dumb woman—because she died in insulin coma five years after the retinopathy had set in A f fth pat ent was excluded because the d abetes had set in before the insul n era

Th s leaves 101 patients males and 49 females all of whom had been on ulin and dietary treatment from the onset of the d abetes Ninety-one pat ents were over 30 years of age when the analysis was con cluded The youngest was ea s and the oldest 65 yea s of age

Table I and II l st the ma tal and social status D abetes mellitus had been d agnosed in the a e range 30 yea s a erage 15 years (F g 1)

[1] Presented at the annual meet ng of the Scand na an Soc ety of the Stud of D abetes Oslo Norway February – 1967

Table I *Matrimonial status of juvenile diabetics presenting retinopathy and/or nephropathy*

Figures in brackets represent number of patients

	Married		Divorced		Unmarried	
No permanent protein uria (53)	76	(40)	11	(6)	13	(7)
Permanent proteinuria (48)	50	(24)	2	(1)	48	(23)
Total (101)	63	(64)	7	(7)	30	(30)
Normal distribution for Copenhagen for ages 30–60 years	77		9		14	

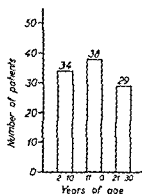


Fig 1 Age of patients when diabetes mellitus was diagnosed

The first ophthalmoscopic sign of diabetic retinopathy was taken to be the appearance of microaneurysms (small red circular dots) The retinopathy was diagnosed by at least two experienced examiners In cases of doubt the uncertainty was settled by the ophthalmological adviser to the Steno Memorial Hospital Professor H Ehlers MD

The first sign of diabetic nephropathy was taken to mean the appearance of intermittent proteinuria without pyuria The proteinuria had to be demonstrated repeatedly within three months and thereafter to be absent for several months Proteinuria was diagnosed either by Heller s test or by the Albustix The qualitative test was always supplemented by a quantitative examination of the urine for protein by the method of Shevky (4) Only urine with 0 1 g or more per l was regarded as positive The ophthalmological examination and the urine analysis were performed on the same day

In 75 patients the first complication had appeared less than 5 years before it was diagnosed since 1 month – 5 years average 2 4 years before the diagnosis of retinopathy and/or nephropathy the patients had shown no signs of these complications Among the remaining 26 patients one had had intermittent proteinuria of unknown duration and 25 a few microaneurysms of unknown duration in one or both eyes It may be considered that on the whole the patients have not had late diabetic manifestations in the form of retinopathy and or nephropathy for more than about two years before they were diagnosed

The patients were followed for 5–20 years average 9 5 years after the first late diabetic manifestation had been diagnosed (Fig 2) One patient could only be followed up to 1959 two only to 1960 and one only to 1961 The remaining patients were last seen in 1962 1964 At the end of the follow up period 31 patients had died All the patients were examined repeatedly in most cases every 3rd month to every other year either on an out patients basis or as in patients usually in the Steno Memorial Hospital The examination comprised a clinical investigation combined with checking of the blood sugar and body weight and examination of the urine for sugar protein and acetone As a rule this was supplemented by ophthalmoscopy determination of the BP in the recumbent position after the patient had rested through the night determination of the ESR and serum creatinine examination of urinary sediment and ECG Chest radiography was done about every third year

Uraemia is defined as persistently elevated serum creatinine above 1 3 mg 100 ml Hypertension is defined

Table II *Occupational status of juvenile diabetic men presenting retinopathy and/or nephropathy*

Figures in brackets represent the per cent distribution

	Skilled	Unskilled
No permanent proteinuria	18	5
Permanent proteinuria	21	8
Total	39 (75)	13 (25)
Normal distribution for Copenhagen	64	36

[a] From the Central Labour Exchange for Copenhagen and Frederiksberg

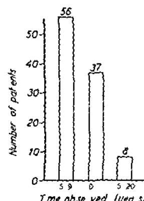


Fig Duration of observation after first symptoms of late diabetic manifestation (retinopathy and or nephropathy)

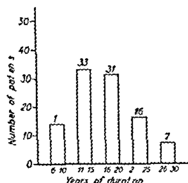


Fig 3 Duration of diabetes before the first late diabetic manifestation was diagnosed

as persistent elevation of the systolic blood pressure beyond 160 mm Hg or of the diastolic blood pressure beyond 100 mm Hg Impaired vision is defined as persistent visual impairment in at least one eye to 6/18 or less

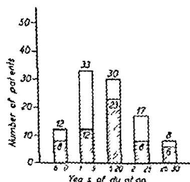


Fig 4 Duration of diabetes before retinopathy and nephropathy was diagnosed The white columns represent number of patients with retinopathy The hatched columns represent number of patients with nephropathy

RESULTS

In 69 patients (68 %) the retinopathy was the first complication in 16 (16%) proteinuria was the first and in 16 (16 %) the retinopathy and proteinuria were diagnosed at the same time

At the end of the follow up period all patients but one had retinopathy However this patient had not been examined by ophthalmoscopy during the five years before death Fifty seven patients showed signs of nephropathy including nine who had only intermittent proteinuria but renal function was preserved judging by the serum creatinine concentration

The first manifestation of late diabetic angiopathy (retinopathy and/or nephropathy) occurred 6–29 years average 16 4 years after the onset of diabetes (Fig 3)

Late diabetic manifestations never appeared before the age of 16 years even though in 34 cases the diabetes had been diagnosed before the age of ten

Retinopathy set in 6–30 years average 16 9 years after the onset of diabetes Nephropathy set in 7–32 years average 17 3 years after the onset of diabetes (Fig 4) It is also apparent from Fig 4 that the nephropathy developed later than the retinopathy

The prognosis for juvenile diabetics after the appearance of the first late diabetic manifestation is illustrated in Fig 5 Ten years after the appearance of retinopathy and/or nephropathy only a little over one third of the patients were alive with normal kidney function and/or no permanent visual impairment

Figs 6 and 7 show the entire group divided

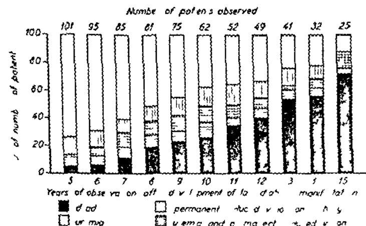


Fig 5 Prognosis for juvenile diabetics after development of late diabetic manifestation

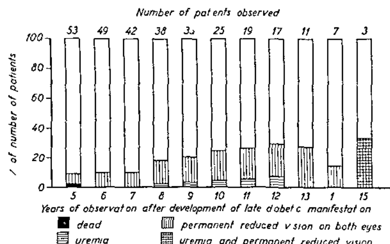


Fig 6 Prognosis for juvenile diabetics without permanent proteinuria but retinopathy (group B)

into two (A) The group of patients who developed persistent proteinuria during the follow up period (B) the group that did not develop persistent proteinuria It is apparent from the figures that the prognosis was highly dependent upon whether persistent proteinuria developed as in group A 43% had died 10 years after the diagnosis of the first complication while in group B there had been no death Table III lists various data which serve to illustrate the two sub groups further

It is apparent that the groups are comparable in respect of size age and duration of diabetes Group A however includes a somewhat larger proportion of men In patients who developed persisting proteinuria the first signs of late diabetic manifestation appeared somewhat earlier The proteinuria was manifest nearly always before or within five years after the first sign of retinopathy had appeared and the duration of intermittent proteinuria was relatively brief Uraemia hypertension and oedema occurred almost exclusively in group A while there did not seem to be any difference in respect to arterio sclerotic manifestations Severe ocular manifestations too were considerably more common in group A It should be mentioned that the incidence of diabetic coma in group A was almost twice that in group B Out of the 30 patients who died in group A five succumbed to coronary occlusion and one to cerebral insult Only two of these patients were uraemic The remaining 24 died of uraemia

The patient of group B died of coronary occlusion

Table IV gives some clinical data for the pa

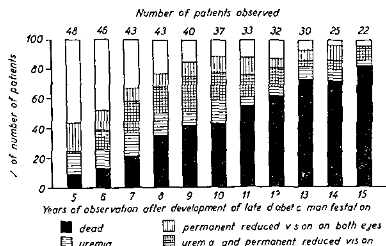


Fig 7 Prognosis for juvenile diabetics with permanent proteinuria (group A)

Table III *History clinical and laboratory data for juvenile diabetics developing persisting proteinuria (group A) and not developing persisting proteinuria (group B)*

	Group A (+ protein uria)	Group B (– protein uria)
No of patients	48	53
Sex (males)	60	43
Age when d m was diagnosed (range)	15 0 y (2 30)	15 4 y (2–30)
Duration of d m	24 9 y (14–41)	27 3 y (15–38)
Family history of d m (parents sisters brothers) in	50	49
First late diabetic manifestation diagnosed after	15 1 y (6–26)	17 7 y (7–29)
First late diabetic manifestation diagnosed within 15 y in	46	32
Proteinuria diagnosed before or within 5 y after retinopathy was diagnosed	9	9
Dead	63	2
Uraemia	83	6
Persistent elevation of ESR	8	4
Hypertension	83	6
Oedema	58	4
Arteriosclerosis (angina pectoris intermittent claudication calcification in vessels of lower limbs)	31	30
Intermittent proteinuria (mean duration and range)	(2 2 y ½–7 y)	17 (> 5 4 y > 2– > 12 y)
Proliferative retinopathy severe bleeding in vitreous body severe glaucoma	58	25
Reduced working ability	68	21 *
Acidotic coma per patient	0 63	0 36

tients who died with persisting proteinuria It will be seen that the symptom complex of severe proteinuria elevated serum creatinine hypertension and oedema must be considered as a very bad omen

Analysis of the case histories revealed that in the majority of those patients who developed per

Table IV *Some characteristic signs and laboratory data for juvenile diabetics who died with persistent proteinuria*

No of cases	30
Elevated ESR	97 *
Proteinuria > 0 5	93 *
Uraemia	93
Hypertension	93 *
Oedema	83 *

Table V *Prognosis of diabetes mellitus in relation to age at onset (of diabetes)*

	Age at onset of d m (y)		
	2–10	11–20	21–30
No of patients	34	38	29
Duration of diabetes (y)	27 5	23 8	27 7
Dead	6	37	28
Uraemia	44	51	3
Permanent proteinuria	47	53	41
Permanent visual impairment	35	37	41
Proliferative retinopathy severe bleeding in vitreous body glaucoma	53	37	38

manent proteinuria the course had been as follows After two years intermittent proteinuria the proteinuria became permanent A year or two later hypertension appeared From the 4th to 5th year after the onset of proteinuria the ESR became persistently elevated A little later oedema became manifest and 6–7 years after the onset of proteinuria the serum creatinine was elevated This is in quite good keeping with Thomsen s observations (6) However individual variations were pronounced From 0–18 years might pass average 6 1 years after the diagnosis of proteinuria until elevated serum creatinine (uraemia) appeared If the proteinuria exceeded 0 2 % 16 of 22 patients succumbed within five years

In Table V the prognostic significance of the patients age at the onset of diabetes is analysed

Like the studies of Keiding et al (2) and of Skouby (4) this analysis also showed a better

Table VI *Prognosis of diabetes mellitus related to time of appearance of late diabetic manifestation*

	Duration of d m until late diabetic manifestations developed	
	15 y	15 y
No of patients	47	54
Duration of diabetes	21 5	29 8
Dead	32	30
Uraemia	52	18
Permanent proteinuria	53	43
Permanent visual impairment	36	35
Proliferative retinopathy severe bleeding in vitreous body glaucoma	43	43

prognosis in respect of life vision and kidney function for diabetics whose disease had set in after the age of 20 It also seems likely that patients who develop diabetes during the first decade of life fare relatively well considering the long duration of the disease

Table VI shows that patients who developed late diabetic manifestations at an early stage of their disease fared worse than patients whose complications developed later

DISCUSSION AND CONCLUSION

The material shows that the prognosis of juvenile diabetes depends primarily upon whether the patients develop persistent proteinuria

If this can be avoided the prospects for life and health are considerably better than previously assumed However the patients who do not develop persistent proteinuria will presumably not enjoy a normal length of life most of them meeting premature death because of arteriosclerotic vascular diseases

As might be expected all the ill luck attends the group of diabetics that develop permanent proteinuria This applies not only physically but also familially and socially this group includes three times as many unmarried persons and three times as many with reduced working capacity as the others

The following appear to be bad prognostic signs in a juvenile diabetic

1 Appearance of proteinuria before or within five years after the diagnosis of retinopathy
2 Occurrence of persisting proteinuria
3 Proteinuria exceeding 0 2%
4 Appearance of retinopathy and/or nephropathy before the diabetes has been present for 15 years
5 Repeated episodes of diabetic coma
6 Onset of diabetes between the ages of 10 and 20 years

In juvenile diabetics there appears to be a correlation between persistent proteinuria and nodular glomerulosclerosis Thomsen (6) found nodular glomerulosclero is in renal biopsies from about 80° of his juvenile diabetics with proteinuria and a duration exceeding ten years while no diabetics without proteinuria exhibited nodular glomerulosclerosis

It is not known why some diabetics develop nephropathy with proteinuria and nodular glomerulosclerosis while others show no clinical signs of nephropathy Probably many factors also immunological play a role in the pathogenesis of diabetic nephropathy

It is to be hoped that further animal experiments will throw more light upon the pathogenesis of nodular glomerulosclerosis Effective treatment is not possible until the pathogenesis of this severe renal disease has been elucidated

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FAMILIAL PROTEIN INTOLERANCE WITH DEFICIENT TRANSPORT OF BASIC AMINO ACIDS

Report on an Adult Patient with Chronic Hyperammonemia

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Abs act An adult patient suffering from prote n in tolerance with deficient transport of basic amino acids is described The early history of the patient was typical of this inborn d sorder of am no acid metabolism. wean in was followed by prolonged diarrhea spontaneous restriction of protein in the diet and by slow growth. Abandonment of the carbohydrate fat diet at the age of 18 years was followed by an increased growth rate and in a very few months by mental changes The timing of these three inc dents suggests a causal relationship between them. It therefore seems that both normal body growth and normal cerebral function are possible in protein intolerance in the indi idual patient however they seem to be mutually exclusive Analogous considera t ons in other inborn metabolic disorders of urea syn thesis ha e been presented (5)

Since the first report on familial protein intoler an e (PI) with deficient transport of basic amino acids (3) this disease has proved to be an inborn metabolic disorted not particularly rare in the Finnish population All the patients presented so far have been children They have lived on a self-chosen low protein diet and their main clinical features have been dwarfish stature and hepato (spleno-)megaly (1 3) In contrast to other in born failures of ammonia detoxification patients suffering from PI have been of average int lli gence and without any neurological manifesta tions of hyperammonemia This feature has been as ribed to the mild degree of the hyperammo-nemia in PI and this in turn principally to the rigid spontaneous exclusion of protein from the diet (1)

We d scribe here an adult patient suffering from PI with a clinical history which differs clearly from those reported earlier The clinical course illustrates the effects of breakdown of protein avoidance in this disorder with concomi tant grave manifestations of chronic recurrent hyperammonemia

CASE REPORT

The patient was the son of a deceased farmer 23 years old on admission One of his younger sisters aged 15 had all her life refused to eat protein rich food the diagnosis of PI was later verified by urine aminophero graphy and by intravenous amino nitrogen load ngs (1 3) Other s blings and the living mother were normal and excreted normal amounts of amino acids

The p rinatal history was uneventful Institution of cow s m lk feeding at the end of the first year of life was followed by prolon ed watery diarrhea and by retar dation of physical development. The abdomen protruded and there were sign of rickets in spite of continued vitamin D prophylaxis

Attendance at school was regular after five years of elementary school the patient, adhering to a self imposed carbohydrate fat diet, was physically clear y underde veloped From the age of 18 years, the patient gradu ally increased the protein intake mainly because of strong pressure from his family During the next two years he used to drink about one l of cow s milk daily and to eat eg s meat, and liver The changes in the diet were followed by periodic pains in the upper abdomen accompanied by vomiting Nevertheless he grew about 0 cm in height during the next four years (Fig. 1) Af ter the change of diet his mental alertness gradually de creased At the age of 19 he had the first attack of stupor Later on these episodes became more frequ nt occurring t pical y n the afternoon or e ening The onset of an attack was always ins d ous and the patient gradu ally becam unc nscious occas onal jerk ng or flapping mo ements of the l mb could be observ ed The attacks lasted fo se eral hours On the morn ng following an attack the patient w s usually able to go to work but somet me complained of headache and lack of appetite

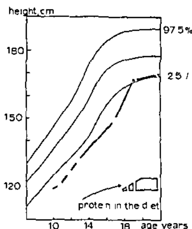


Fig 1 Height of the patient at different ages The commencement of a protein containing diet is expressed schematically

At the age of 20 he was admitted to hospital because of recurrent abdominal pain and episodes of stupor Clinically the most remarkable finding was pronounced hepatomegaly EEG examination revealed low voltage reduced alpha activity and some episodic low voltage theta activity

The condition of the patient deteriorated with time and he was readmitted two years later He was now definitely mentally retarded The abdominal pains had persisted and because the gall bladder could not be visualized radiologically cholecystectomy was performed The organ was found to be normal and was removed At operation gross hepato and splenomegaly was noted Postoperatively the abdominal complaints remained unchanged and the patient was transferred to a mental ward because of the ever worsening stuporous attacks At this time the EEG showed a generalized irregular 3–5 cps activity (Fig 2) There was no fluctuation of the activity as during normal sleep A slight increase of the frequency was seen during hyperventilation and photo stimulation

Five days after this recording the patient was admitted to the University Central Hospital in Helsinki

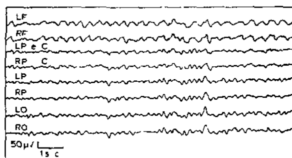


Fig EEG recording taken during mental symptoms on an ordinary diet

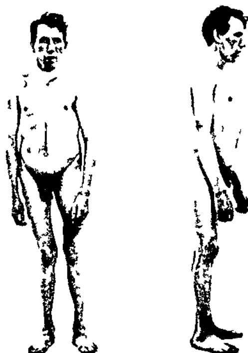


Fig 3 The patient on admission

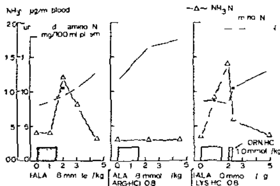
Clinical findings

The patient was 169 cm tall and weighed 64 kg He was oriented and answered questions slowly He walked with flexed knees and there was coarse tremor in the hands *The skin was pale warm and moist The thoracic spine* was scoliotic The firm smooth and non tender liver edge was 5 cm palpable when the patient was supine The external genitals were masculine and the pubic hair was feminine in distribution (Fig 3) Muscular power was subnormal

The patient was given the ordinary diet of the ward On the second day he became stuporous about three hours after a protein rich (milk and beefsteak) meal Simultaneously the tremor in the hands grew worse and the tendon reflexes in the extremities were exaggerated He was unable to move and vomited repeatedly Five hours after the meal he was unconscious and no reaction to painful stimuli was observed The patient was amnesic of the attack on the following day Blood ammonia concentration taken six hours after the meal was 3? μg/ml of blood

After this episode the dietary protein was reduced to a minimum Blood ammonia was checked repeatedly and all fasting values were in the normal range No comatose periods or stupor appeared during the next five weeks in the ward The patient complained of irregular pains in the right upper abdomen but no definite cause could be found for them Arginine HCl given orally in g portions neither prevented the pain nor abolished it He returned home on a low protein diet

When seen four months later he complained of som weakness in the limbs there had been no further attacks



Fi 4 The am no nitrogen tolerance tests Blood am monia plasma urea and amino nitrogen courses are typical of PI (1)

of unconsciousness or of abdominal pain There was no impro ement in the mental state

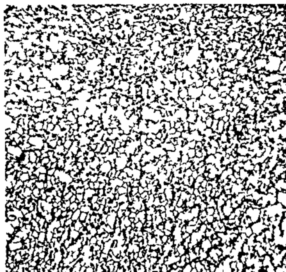
Laboratory data

Routine tests of blood and urine were normal Occasional WBC counts below 4000/mm were recorded Massive lysinuria and low plasma lysine and arginine levels were detected in high voltage electrophoresis Tests for liver funct on were normal except the combined intravenous alcohol galactose tolerance test (4) its result pointed to fatty degenerat on of the l ver Exocrine pancreas func tion was normal as shown by the results of a duodenal intubation study In a series of intravenous amino nitrogen loading tests (1 3) an impairment of urea synthesis typical of PI was establi hed (Fig 4)

In X ray stud es marked flattenin and biconcav ty of the vertebral bodies were noted along the whole length of the spine (Fig 5) The cristal epiphyses were unossif ed indicat ng a retarded bone age



Fig 5 X ray picture of the lumbar spine The vertebral bodies are flattened and bi concave simulat ng extreme osteoporos s but the calc um content of the bone was re garded as normal



F g 6 Fatty degeneration in the liver biopsy specimen In the upper part of the figure normal liver structure is seen H E × 170

Liver biopsy specimens taken during cholecystectomy revealed patchy fatty degeneration (Fig 6)

EEG tracings were obtained during a period of nor mal blood ammonia concentration (two weeks after the record ng in Fig 2) and during hyperammonemia induced by the alanine lysine load The former recording showed a normal 10 cps alpha rhythm with occipital predomi nance One hour after the beginning of the amino acid infusion the alpha activity was clearly reduced in spite of efforts to keep the patient fully awake At the same time the patient began to complain of a constant pain in the abdomen. Twenty minutes after the end of the nfusion the patient was disoriented Infus on of 1-orni thine HCl was then commenced and in 10 minutes the alpha activity again increased The ornithine infusion lasted for 0 minutes and immediately thereafter the pa tient was symptom free

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BODY COMPOSITION IN HEART DISEASF

Total Exchangeable Electrolytes in Hyponatraemic Hypochloraemia

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Abstract In order to define the alterations in body composition in cardiac patients with hyponatraemic hypochloraemia, the chloride space total exchangeable chloride sodium space total exchangeable sodium and total exchangeable potassium were measured in 12 subjects with hyponatraemic hypochloraemia and in 1 pa tients with normonatraemia The groups were selected to be comparable with regard to average body weight, mean age and sex distribution

Statistical analysis defined the pattern of body com position in the hyponatraemic hypochloraemic group in the light of the findings in the normonatraemic group as follows 1 A significant and parallel decrement of serum chloride and serum sodium concentrations 2 An un changed chloride space and sodium space 3 A parallel decrement of total exchangeable chloride and total ex changeable sodium although not statistically significant 4 A normal or increased total exchangeable chloride and sod um in relation to predicted normal values 5 A normal distribution of sod um in relation to chloride space and 6 A significant rise in serum potassium con centration and a tendency to decrease of total exchange able potassium

Whil the interrelations between chloride space and sodium space and between total exchangeable chloride and total exchangeable sodium were the same in the two groups, the ratios of Cl /Na and Na /Cl space were signif antly lowered in the hyponatraem c hypochloraem group

It is concluded that although it is likely that a deficit of total exchangeable chloride in relation to total ex chan able sodium associated with metabolic alkalosis and decreased total exchangeable potassium may ac count in part for the hypochloraemia seen in both groups the major cause of the marked hypochloraemia in the h ponatraemic group is apparently a dilut on of chloride in an expanded extracellular phase caused by the water retention responsible for the hyponatraemia

Hypochloraemia is a common finding in patients with heart disease and may occur with or with out relation to previous diuretic treatm nt (5 12 13)

The low serum chloride seen in patients with normal serum sodium concentration is most often induced by diuretics (12 13) Isotope dilutional studies in cardiac patients with normonatraemic hypochloraemia have defined the characteristic alterations in body composition as a deficit of total exchangeable chloride in relation to total ex changeable sodium while the chloride and sodium spaces remain virtually the same Associated find ings are metabolic alkalosis and a tendency to deficit of total exchangeable potassium (11)

The lowest values for serum chloride concen tration in heart disease are found however in patients with hyponatraemia and may occur with or without previous diuretic treatment (5 8 12) The characteristic alterations in body composi tion in hyponatraemic hypochloraemia have not yet been defined Since such patients often present severe problems in terms of treatment a study of the basic changes in body composition is highly desirable

It will be the purpose of this paper to examine the interrelations between chloride space sodium space total exchangeable chloride total exchange able sodium and total exchangeable potassium in cardiac patients with hyponatraemic hypochlor aemia and to compare with the finding in normo-natraemic patients

MATERIAL AND METHODS

The material includes 4 patients with heart d sease 16 males and e ght females The clin al d agnoses the degree of con est e heart fa lure and the treatment a pl ed a e shown in Tables I and II

The material is d vided into two parts accord ng to the serum sod um concentrations in order to contrast the find ngs n the group with hypona raem a (below 134 mEq l) and the group w th normal serum sodium con cent at on (> 136 mEq l) For purposes of comparison

Table I *Clinical and laboratory data*

Case no	Sex	Diagnosis	Oedema	Treatment	Age (y)	Body weight (kg)	(Na)s (mEq/l)	(Cl)s (mEq l)	(K)s (mEq l)	Serum standard bicarbonate (mEq l)
Hyponatraemic group										
1	o	ASHD	−++	D C S	48	57 5	123	70	3 1	25 0
2	o	CP	+++	D C S	40	99 4	128	90	4 5	23 1
3	♂	ASHD	++	D F S	52	49 2	133	86	4 3	27 9
4	♂	MI	++	D C S	56	71 8	131	95	5 [illegible]	23 5
5	o	MI	−+	D F S	4?	60 0	134	91	4 3	[illegible]4 9
6	♂	MYO	+++	D C	39	73 5	134	87	4 0	25 3
7	♂	MS	0	D C S	59	70 3	121	87	6 4	22 9
8	♂	ASHD		D F S	56	84 0	133	83	5 0	24 0
9	♀	MS	+++	D C	50	57 7	128	79	5 0	2 4
10	♀	MI	++	C S	49	52 8	127	89	5 5	[illegible]1 0
11	♀	ASHD	+	C S	54	65 4	133	94	5 1	24 6
12	♀	MI	++	D C S Th	52	48 3	117	74	5 0	21 3
Mean value					49 7	65 83	128 5	85 4	4 78	23 8
S E						4 30	1 6	1 9	0 24	0 59
Normonatraemic group										
1	o	CA	−++	D C	48	68 3	137	100	3 7	2? 9
2	o	CP	0		41	85 6	141	100	4 4	25 2
3	o	ASHD	+	D C	50	58 5	14[illegible]	101	4 1	27 5
4	♂	AI	+++	D Th	64	75 8	141	97	3 7	21 4
5	o	MS	0	D C	39	62 4	141	95	4 6	24 5
6	♂	CP	−+	D C S	28	67 0	140	100	3 8	21 8
7	o	ASHD	++	D F S	57	66 8	138	95	4 [illegible]	[illegible]4 0
8	♂	CP	0		44	87 6	144	104	4 [illegible]	22 1
9	♀	MS	+		57	46 3	138	99	4 6	2[illegible]1
10	♀	ASHD	−+	C	58	57 3	145	96	4 6	27 0
11	♀	MS	−++	D Chl Th	56	46 5	140	85	4 4	30 0
12	♀	ASHD	+++	Chl	54	76 5	14[illegible]	93	3 4	26 0
Mean value					49 7	66 55	140 8	97 1	4 14	24 5
S E						3 92	0 7	1 4	0 12	0 6[illegible]

AI = aortic incompetence ASHD = arteriosclerotic heart disease CA = coarctation of the aorta CP = constrictive pericarditis. MI = mitral incompetence MS = mitral stenosis MYO = myocarditis D = digitalis C = cyclopenthiazide Chl = chlorothiazide F = furosemide S = spironolactone Th = thiomerin

S E = standard error of the mean

normal = measured value as of predicted normal values (ee Definitions)

the hyponatraemic and the normonatraemic groups have the same sex distribution and the same mean a e and mean bo ly wei ht

The measurements of chloride space total exchangeable chloride sodium space total exchangeable sodium and total exchangeable potassium are carried out by the use of the isotopes Br^{82} Na^{24} and K^{42} according to methods previously described (9) The serum sodium potassium chloride and standard bicarbonate concentrations are determined according to methods previously reported (13)

Conventional statistical methods are used (14)

Definitions Derived Values and Abbreviations

(Cl)s = serum chloride concentration (mEq/l)

(Na)s = serum sodium concentration (mEq/l)

(K) = serum potassium concentration (mEq/l)

Cl space = chloride (bromide) space (litres) Cl space represents the theoretical volume of dilution of chloride (bromide) if chloride (bromide) is distributed throughout in the same concentrational relationship as in plasma The free and complete exchange of bromide[82] and chloride is assumed

Cl = total exchan eable chloride (mEq)

Na space = sodium space (l tres) s e definition of Cl space

Na = total exchangeable sodium (mEq)

ECNa = total extracellular sodium (mEq) which is derived from the equation

ECNa = (Na)s × (Cl space) × 0 89

Residual Na = residual sod um (mEq) i e the amount of Na not accounted for as total extracellular sodium is obtained from the formula

Residual Na = Na − ECNa

K = total exchangeab e potassium (mEq)

The values obtained for total exchangeable ele trolytes are also expressed as percenta es of predicted normal values for each individual The predicted normal values take into account th effect upon body composition of

Table II *Body composition data*

Case no	Na space (l)	Na (mEq)	Na_e normal	Residual Na (mEq)	Cl space (l)	Cl (mEq)	Cl normal	Na /Cl space (mEq l)	Cl /Na space (mEq/l)	K_e (mEq)
Hyp t aem c g oup										
1	33 0	4070	155	310	34 3	2400	1 5	119	73	1650
2	49 8	6370	163	00	54 2	4880	171	118	98	2880
3	25 5	3390	149	480	24 6	21_0	1 8	138	83	1670
4	31 4	4110	134	410	31 6	3010	134	130	96	2470
5	23 1	3080	115	230	2 9	2160	111	134	94	_00
6	35 0	4690	143	3 0	36 8	3200	133	127	91	1830
7	22 2	_680	89	180	24 3	2120	97	110	95	_150
8	5	3340	98	450	25 3	2090	84	132	83	_890
9	27 0	3460	154	150	29 0	2790	140	119	85	1030
10	_ 8	_890	120	470	21 4	1900	109	135	83	16_0
11	22 0	29 0	11	450	_0 9	2070	109	140	94	300
12	19 5	280	118	300	19 0	1410	102	1 0	72	1080
Mean value	28 04	3607	129 2	3 9	8 69	2471	1 0 3	1 6 8	87 3	1977
S E	_41	317	6 9	35	2 81	258	6 6	2 8	2.5	176
No monat a mic g o p										
1	33 1	4530	153	710	31 4	3140	145	144	95	030
2	30 5	4300	123	590	29 5	_950	114	146	97	3560
3	27 1	3850	138	310	_8 0	2810	134	138	104	2490
4	41 1	5800	181	440	42 6	4140	177	136	101	1820
5	21 8	3070	111	300	22 1	2100	104	139	96	2740
6	31 9	4470	154	60	35 4	3540	167	1 6	111	25 0
7	25 0	3450	118	_90	6 6	25 0	118	130	101	2180
8	29 8	4790	114	720	28 7	2970	116	149	100	3350
9	17 8	2450	107	290	17 6	1740	104	139	98	1730
10	22 5	3260	148	440	21 8	2100	131	150	93	1670
11	0 7	2760	142	210	20 4	1730	1_3	135	84	1500
1	40 0	5680	210	340	43 3	4030	04	131	101	24 0
Mean value	28 44	3993	141 6	39	28 95	2814	135 6	138 6	98 4	334
S E	13	307	8 8	57	2 42	_36	9 1	2 1	1 7	188

Abbreviat ons as in Table I

sex, a e and a tual body we ht and are based upon the data for normal body composition given by Moore et al (8) Since no correction was used for the weight increase caused by oedema th predicted normal values will tend to be too high and the valu s expressed as percentages of normal values will tend to be underestimated (9)

RESULTS

The data for body composition of the hyponatraemic and the normonatraemic groups are listed in Tables I and II The results of statistical analyses are shown in Tables III and IV

A consid ration of mean results and of cor relative analysis leads to the following conclu sions

I *Body Weight Age and Sex*

As shown in Table I the hypo and normonatraemic groups were comparable with regard to mean body weight average age and sex distri bution This similarity was int nded in order to minimize the effect of these variables upon body composition (8 9)

II *Sodium*

a $(Na)_s$

According to the selection of the material the mean values for serum sodium concentration were significantly different in the hyponatraemic group (128 5 mEq l) and in the normonatraemic group (140 8 mEq l)

b *Na space*

The Na space amounted to 28 0 l in the hypo natraemic group and to 28 4 l in the normo natraemic group This difference was not statisti cally significant

Table III *Statistical summary of body composition in hyponatraemic and normonatraemic groups*

Mean values ± s e Weight (kg)	$(Na)_s$ (mEq/l)	Na space (l)	Na (mEq)	Residual Na (mEq)	Na_e/Cl space (mEq/l)	$(Cl)_s$ (mEq/l)	Cl space (l)	Cl_e (mEq)	Cl_e/Na space (mEq/l)	$(K)_s$ (mEq/l)	K (mEq)	Standard bicarb (mEq/l)
Hyponatraemic group												
65 83	128 5	28 04	3607	329	126 8	85 4	28 69	2471	87 3	4 78	1977	23 83
±4 30	±1 6	±2 41	±317	±35	±2.8	±1 9	±2 81	±258	±2 5	±0 24	±176	±0 59
Normonatraemic group												
66 55	140 8	28 44	3993	392	138 6	97 1	28 95	2814	98 4	4 14	2334	24 54
±3 92	±0 7	±2 13	±307	±57	±2.1	±1 4	±2 42	±236	±1 7	±0 12	±188	±0 62
Difference												
−0 7.	−12 3	−0 40	−386	−63	−11 8	−11 7	−0 26	−343	−11 1	+0 64	−357	−0 71
±5 80	± 1 7	±3 19	±436	±67	± 3 5	± 2 4	±3 70	±346	± 3 0	±0 27	±257	±0 74
Significance of difference (*p* value)												
—	<0 001	—	—	—	<0 005	<0 001	—	—	<0 001	<0 05	—	—

c Na_e

In accordance with the above results the mean value for Na was lower in the hyponatraemic group (3607 mEq) than in the normonatraemic group (3993 mEq) This difference was not statistically significant

It is noteworthy that the hyponatraemic group showed a mean value of 129% for Na_e expressed as per cent of predicted normal values (range 89–163%) The corresponding results for the normonatraemic group were 141% with a range from 107 to 210% Since the normal range is 80–120% it appears that the hyponatraemia cannot be related to an absolute deficit of sodium as Na was either normal or increased In all edematous hyponatraemic patients Na_e was increased above predicted normal values

d *Distribution of* Na_e

The total extracellular sodium amounted to 3278 mEq in the hyponatraemic group and to 3601 mEq in the normonatraemic group

The residual Na i e the amount of Na not accounted for by total extracellular Na represented 329 mEq in the hyponatraemic group and 392 mEq in the normonatraemic group This difference was not statistically significant This finding implies that if a corrected chloride space is accepted as a measurement of total extracellular water the hyponatraemia cannot be related to a storage of exchangeable sodium outside the extracellular phase

III *Chloride*

a $(Cl)_s$

Serum chloride levels range from 85 to 101 mEq/l in the normonatraemic group and from 70 to 95 mEq/l in the hyponatraemic group The mean values of 97 1 mEq/l and of 85 4 mEq/l are significantly different It is noteworthy that the difference between the serum chloride levels of the two groups is closely similar to that of the serum sodium levels (Tables I and III)

b *Cl space*

The mean values of the chloride space are closely similar in the hyponatraemic (28 7 l) and normonatraemic group (29 0 l)

Table IV *Summary of regression analysis*

Group	Measurement y	Reference x	No	Correlation coefficient (r)	Significance of correlation (p value)	Regression equation	s.d
Hyponatraemic	Cl space	Na space	12	0 997	0 001	Cl space = 1 16 (Na space) − 3 8	±1 1
Normonatraemic	Cl space	Na space	12	0 983	< 0 001	Cl space = 1 10 (Na space) − 2 2	±1 6
Hyponatraemic	Cl_e	Na	12	0 968	0 001	Cl_e = 0 79 (Na) − 378	±221
Normonatraemic	Cl_e	Na_e	12	0 985	0 001	Cl_e = 0 76 (Na_e) − 09	±157

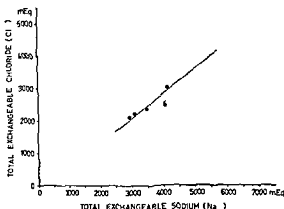


Fig 1 Relation between Cl and Na in hypo- and normonatraemic heart disease The lines indicate the re ression line (±2 S.D) for the normonatraemic group The dark dots show the values for the hyponatraemic group The similarity in slope and adjusted means is evident

c *Cl*

In accordance with preceding results the Cl was lower in the hyponatraemic (2473 mEq) than in the normonatraemic group (2814 mEq) The dif ference was not statistically significant

In relation to predicted normal values the Cl of the hyponatraemic group showed a mean value of 120 with a range from 84 to 171% while the normonatraemic group had a mean value of 136 with a range from 104 to 204° Ap parently the hypochloraemia is usually not related to an absolute deficit of chloride

IV *Interrelations Between Cl and Na*

a *Cl and Na_e*

While (Cl)s and (Na)s are moderately significantly correlated in the total series ($r = 0\ 74$ $p < 0\ 001$) the Cl and Na_e are highly significantly correlated in both subgroups as shown in Table IV

In covariance analysis of the regressions of Cl on Na_e no significant difference is found in slopes or in adjusted mean between the hypo- and normonatra mic groups This result indicates that the more pronounced hypochloraemia in the hy ponatraemic group cannot be related to a deficit of chloride in relation to sodium in excess of that present in the normonatraemic group (Fig 1)

b *Cl space and Na space*

Within each subgroup the Cl space and Na space are highly significantly correlated ($r = 0\ 98$ $p <$ 0 001) and in covariance analysis no significant difference is found in slopes or in adjusted means (Table IV) This finding suggests that sodium and chloride are mainly found together and is most easily explained by the assumption that both electrolytes are present in an expanded extra cellular phase (Fig 2)

c *The ratios Cl /Na space and Na /Cl space*

As shown in Tables II and III there is an almost parallel decrease in these ratios from the normo natraemic to the hyponatraemic group The sig nificant decrease in Cl /Na space is compatible with the interpretation that hypochloraemia is caused by a dilution of chloride in an expanded extracellular phase Similarly the decrease of Na /Cl space suggests a dilution of sodium in an excess of extracellular water

V *Potassium*

a *(K)s*

The mean value of (K)s was significantly higher in the hyponatraemic (4 78 mEq/l) than in the normonatraemic group (4 14 mEq/l)

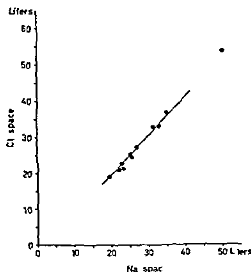


Fig Relat on between Cl space and Na space in hypo and no monatra mic heart d sease The lines indicate the egre sion l ne (± S.D) for the normonatraemic group The dark dots indicate the values for the hyponatraemi group The similarity n slope and in adjusted means is evident

b K_e

The total exchangeable potassium had a mean value of 1977 mEq in the hyponatraemic and of 2334 mEq in the normonatraemic group Significance of difference was not achieved

VI *Acid Base Balance*

The standard bicarbonate values were normal or slightly increased in both groups The mean values were not significantly different

DISCUSSION

The hyponatraemic and normonatraemic groups were selected to be comparable with respect to age sex and body weight in order to minimize the effects of these variables on body composition (8 9) Consequently the mean values for total exchangeable electrolytes and for volumes of dilution of isotopes should be directly comparable However *since the varying degree of oedema and* of wasting tends to increase the standard deviations of the body constituents and to diminish the statistical significance levels of differences ratio and regression analysis was also used

Although the hyponatraemic and normonatraemic groups according to the selection show a significant difference in serum sodium levels it appears that the Na spaces are closely similar The total exchangeable sodium value is slightly but not significantly lower in the hyponatraemic than in the normonatraemic group However in both groups the Na is normal or increased above predicted normal values Therefore it appears that hyponatraemia cannot generally be ascribed to an absolute deficit of sodium Actually in all oedematous hyponatraemic patients the Na_e is definitely increased above predicted normal values as found in other studies (2 4 8 10)

As shown in Table III the Cl spaces of the two groups are closely similar and nearly equal to the mean values of the Na spaces When it is assumed that chloride is essentially an extra cellular electrolyte (9) it appears that the total exchangeable sodium in the hypo and normo natraemic groups can largely be accounted for as total extracellular sodium alone As shown in Table III the residual sodium i e the amount of Na_e not accounted for as extracellular sodium is slightly lower in the hyponatraemic than in the normonatraemic group Consequently this study does not support the contention that hyponatraemia is caused by a storage of sodium outside the extracellular phase (6 7 15) unless it is assumed that this store of sodium is not exchangeable with isotope This possibility seems remote however The fact that the ratio of Na_e/Cl space is significantly lower in the hyponatraemic than in the normonatraemic group suggests that the hyponatraemia is due to a dilution of sodium in an expanded extracellular phase

The alterations in body composition in the hyponatraemic group include an elevated serum potassium concentration and a tendency to decrease of total exchangeable potassium The latter finding may reflect either a cellular potassium depletion or a reduction of total cellular mass caused by long standing severe illness or a combination of both The combination of hyponatraemia increased Na expanded extracellular phase decreased K and an elevated serum potassium con*centration is the characteristic pattern of body* composition in hyponatraemic cardiac oedema (2 8 10) While the quantitative role of the decreased total exchangeable potassium for the development of hyponatraemia is not yet clearly established it is apparent that the pathogenesis of the hyponatraemic state includes a water retention (2 8 10) and it would appear likely that this water excess also plays a role in the development of the hypochloraemia

The serum chloride levels are below the normal range in several normonatraemic patients listed in Table I The subjects present the findings characteristic of normonatraemic hypochloraemia induced by diuretic treatment a deficit of chloride in relation to total exchangeable sodium associated with potassium deficit and metabolic alkalosis (11) It appears likely that a similar degree of chloride depletion in relation to sodium may be present in some patients with hyponatraemic hypochloraemia

However that a deficit of chloride in relation to sodium is not the major cause of the more marked hypochloraemia in the hyponatraemic group appears from the following observations 1 The average decreases of serum chloride and serum sodium concentrations from the normonatraemic to the hyponatraemic groups are closely similar (Table III) 2 The differences between Cl and Na in the two groups are nearly

equal (Table III) and 3 The relationship Cl / Na is not significantly different in the two groups (Table IV) Since Cl is normal or increased in relation to predicted normal values an absolute chloride deficit cannot be invoked as the cause of the hyponatraemic hypochloraemia Nor would it appear likely that a storage of non-exchangeable chloride outside the extracellular phase can explain the hypochloraemia (3) However the findings of a significantly lower ratio of Cl_e/Na space in the hyponatraemic than in the normonatraemic group strongly suggest that the major cause of hyponatraemic hypochloraemia is a dilution of chloride in an expanded extracellular phase

Apparently the hypochloraemia in the presence of hyponatraemia in cardiac disease is largely accounted for by the same mechanisms as cause the hyponatraemia As mentioned above the hyponatraemia in cardiac oedema must always involve a retention of water (2 8 10 17) The excess of water which dilutes the extracellular as well as the intracellular phases reflects an alteration in kidney function causing a decreased capacity for excretion of free water Possible mechanisms involved are (*a*) a resetting of the osmostat caused by intracellular hypotonocity (potassium loss) (4 16) (*b*) increased secretion of antidiuretic hormone (15 16 17) (*c*) excessive proximal tubular reabsorption of glomerular filtrate with decreased supply of water and solutes to distal tubulus (1 16) and (*d*) interference with the dilutional capacity of the kidney through inhibition of distal tubular sodium reabsorption caused by diuretics (12) The relative significance of these mechanisms for the development and maintenance of the hyponatraemic hypochloraemic oedematous syndrome in cardiac disease remains to be determined

ACKNOWLEDGEMENT

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QUANTITATIVE ESTIMATION OF CELLS IN URINE

An Evaluation of the Addis Count

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Abs act The Addis count, or number of cells excreted in the urin in a fixed time has been assumed to give the best estimate in quantitative determinations The method is based on the assumption that an inverse re lationship exists between the number of cells per unit vo ume and the diuresis

Som of the factors involved in the procedure were studied in a material comprising 75 males without urinary tra t diseases There was no correlation between the number of erythrocytes *per* unit volume and the volume in whi h they were excreted, a slight negative correla tion being found between th number of white cells per un t volume and the diuresis This observation lends some su port to the hypothesis that under normal con ditions erythrocytes enter the urine through the glomer uli white cells being sloughed off the lining of the urinary tract

In the d termination of excretion rates the random variability of haemocytometer counts may cause great variations while the diuresis, under standardized condi tions, shows small variations At high diuresis the con centration of the urine as reflected in the specific rav ties, becomes so low that hypotonic haemolysis may occur The preci ion of results expressed as excretion rates is unknown, because the arithmetic applied makes t impossible to evaluate the precision of the main variable the haemocytomet r count In the author s o inion the method ought therefore to be abandoned The cells *shou d be counted in 1 mm³ of urine and the* resu t expressed as number of cells per mm the mag n tude of the theoreti al error due to the random distri bu ion of cells in the haemocytometer being approxi mately known.

Determination of the excretion rate of cells in urine or number of cells excreted per unit of time was first performed by Hottinger (9) but the method is usually attributed to Addis (1) and often referred to as the Addis count The method is based on the assumption that the excretion of cells is relatively constant, and that the concen tration of cells (number of cells per unit volume) in urine changes in the same way as other con stituents leading to an inverse relationship be twe n cell concentration and the volume of urine excreted (2) However Brosig et al (3) have em phasized that it is not known whether the number of cells excreted p r unit of time (minute hour day) is constant or varies with the volume of the urine

Long collecting periods would serve to elimi nate the influence of diurnal fluctuations (1) but have several disadvantages (10 19) and shorter collecting periods have been recommended (8 10 12) A comparative study of excretion rates in overnight specimens and specimens collected during two hours in the morning revealed no conclusive differences (7) The problems involved in collection of urine have b en discussed exten sively by Addis (2)

Determination of the excretion rate has been assumed to be of particular value in distinguishing between normal and low but pathologically in creased numbers of cells in urine

This paper presents an account of the influence of some of the factors involved in the method

MATERIAL AND METHOD

The material consisted of 75 male patients with no history of urinary tract disorders at any time They had been admitted to hospital with diagnoses that includ d various d spept c disorders (ulcers constipation) neuroti manifestations (especially cardiac neurosis) lumba o or sc atica None of them had fever or used drugs Routine examination of their urine had revealed no patholog cal findings Their age ran ed from 14 to 63 years, averaging 36 4 years

The patients had their last meal at about 6 p m on the day before the investigation and after this were told not to dr nk before the in estigation had been terminated on the next day After cleaning the urethral orifice with

Table I *Rate of urine flow specific gravity pH concentrations (cells/mm³) and excretion rates per hour of erythrocytes and leucocytes in uncentrifuged and centrifuged urines from 75 males without urinary tract disorders*

	Range	Mean	S D
Volume (ml/hour)	17 5–200	45 0	24 82
Specific gravity	1 009–1 030	1 0198	0 00523
pH	5 4–7 5	6 1	0 54
Erythrocytes			
uncentrifuged			
/mm³	0–11 6	1 1	1 61
/hour	0–5 0 313	54 285	89 280
centrifuged			
/mm³	0 2–10 5	0 7	1 34
/hour	578–474 609	34 275	65 974
Leucocytes			
uncentrifuged			
/mm³	0 3–10 5	2 7	2 09
/hour	19 564–468 750	112 546	88 383
centrifuged			
/mm³	0 1–8 4	1 4	1 40
/hour	3 750–66 60?	58 890	55 974

saline urine collected from 8 a.m to 10 a.m was voided in sterile Erlenmeyer flasks

The specimens were examined about two hours after voiding The volume was measured and specific gravity and pH determined After cautious shaking for half a minute specimens were transferred to the two chambers of a Fuchs Rosenthal haemocytometer The number of erythrocytes and leucocytes (including non squamous epithelial cells) or white cells was counted in the total area of the ruled field in both chambers representing a total volume of 6 4 mm

Simultaneously 10 ml of urine was transferred to a graduated centrifuge tube and centrifuged for five minutes at ?00 rpm (radius 16 cm) Nine ml of the supernatant was pipetted off and the sediment was resuspended in the last 1 ml at the bottom of the tube by means of a capillary pipette into which the sediment was drawn ten times The number of cells per unit volume which was assumed to have been increased ten fold was counted in the same Fuchs Rosenthal haemocytometer

The excretion rates based on cell counts in uncentrifuged and centrifuged specimens were calculated and the results analysed by common statistical methods (The statistical calculations were performed by Erna Kamm Bergen)

RESULTS

Range mean values and standard deviations of urine volumes excreted per hour specific gravities pH number of erythrocytes and leucocytes (including non squamous epithelial cells) per

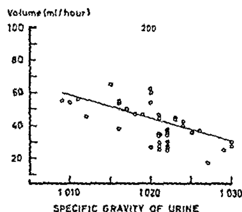


Fig 1 Rate of urine flow compared with the specific gravity of the urine

mm³ (concentration) in uncentrifuged and centrifuged urine as well as their excretion rates per hour are recorded in Table I

There was a significant difference between the number of cells per unit volume in uncentrifu ed and centrifuged urine (erythrocytes $t = -7\ 60$ $P < 0\ 1$%, leucocytes $t = -9\ 84$ $P < 1$%) indicating a significant loss of cells during centrifugation

The correlation between the specific gravities and the rate of urine flow was slightly negative ($r = -0\ 2937$ Fig 1)

No correlation existed between the erythrocyte concentration in uncentrifuged urine and the specific gravity ($r = -0\ 0309$ Fig 2) and the correlation between the leucocyte concentration in uncentrifuged urine and specific gravity was very slight ($r = 0\ 1529$ Fig 3)

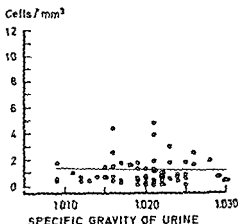


Fig 2 Number of erythrocytes per mm measured in uncentrifuged urine compared with the specific gravity of the urine

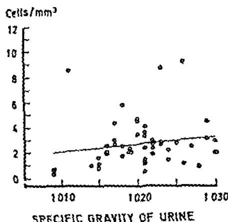


Fig. 3. Number of leucocytes (including non-squamous epithelial cells) per mm³ measured in uncentrifuged urine compared with the specific gravity of the urine.

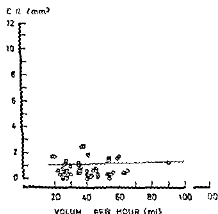


Fig. 4. Number of erythrocytes per mm³ measured in uncentrifuged urine compared with the rate of urine flow (ml/h).

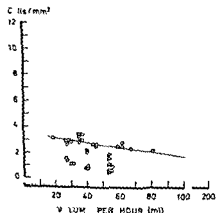


Fig. 5. Number of leucocytes (including non-squamous epithelial cells) per mm³ compared with the rate of urine flow (ml/h).

Correspondingly there was no correlation between the erythrocyte concentrations in uncentrifuged urines and the volumes in which they were excreted ($r = 0.0421$, Fig. 4), although a slight negative correlation was found between the leucocyte concentrations in uncentrifuged specimens and the volumes in which they were excreted ($r = -0.2038$, Fig. 5).

DISCUSSION

In the present study the calculated excretion rates of cells in urine from males without urinary tract disorders, based on counts in centrifuged specimens, agreed fairly well with results reported in the literature, where centrifuged specimens have most commonly been used. The calculated excretion rates in uncentrifuged specimens showed higher values, confirming previous observations that centrifugation causes loss of cells (5).

Discrepancies in reports on excretion rates in normal persons (5) have been related to differences in technique, sex and age (12). The present study revealed *no* relationship between age and number of cells per unit volume (erythrocytes $r = -0.0813$, white cells $r = -0.0403$).

Addis (2) recommended that specimens should be concentrated and acid without, however, stating any limit for an acceptable specific gravity. The lowest specific gravities noted in the present series were 1.009 in three specimens and 1.010 in two. Extensive lysis of erythrocytes in prepared urines occurs at specific gravities below 1.009 (7), while leucocytes are reported to be more resistant. In the present series severe lysis of cells due to hypotonic environments may therefore probably be disregarded. The highest pH in one specimen was 7.5, two of them had a pH of 7.1, and the rest of the specimens showed acid reactions.

At the same level of solute output the specific gravity of the urine is inversely proportional to the volume excreted (23). In the present study the relationship was found to be loose (Fig. 1), in agreement with observations made by Sharp (21).

In conformity with other observations (22) no relationship was found between the concentration of erythrocytes in uncentrifuged specimens and the specific gravity of the urine (Fig. 2) and the relation between the concentration of white cells

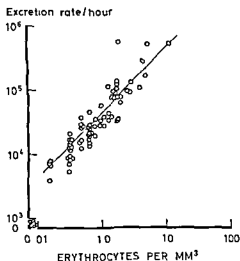


Fig 6 Relationship betw en the number of erythrocytes per mm measured in uncentrifu ed urine and the ex cretion rate per hour

and the specific gravity was found to be very slight (Fig 3) Nor was there any relationship between the concentration of erythrocytes in un centrifuged specimens and the volume in which th y were excreted (Fig 4) although a slight negative correlation was found between the con centration of white cells and the volume excreted per hour (Fig 5) This observation may possibly have some relation to the still unsolved question of how and where these cells enter the urine under normal conditions (18) Microscopical haematuria has been attributed to minimal lesions

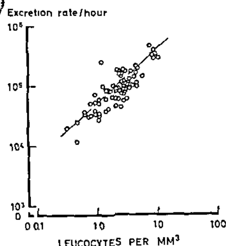


Fig 7 Relationship between the number of leucocytes (including non squamous epithelial cells) per mm meas ured in uncentrifuged urine and the excretion rate per hour

in daily life analogous to the haematuria observed during strenuous sports lengthy standing or even mental exaltation (13 20) in which venous con gestion has been assumed to play a part (3) If the glomerulus is the port of entry the erythro cytes may be subject to hypotonic haemolysis at the thin segment of the loop of Henle Under these conditions no relationship between the erythrocyte concentration and diuresis can be expected

Leucocytes and non squamous epithelial cells have been assumed to be sloughed off from the lining of the urinary tract as a result of normal wear and tear (18) The pres nt observations ap pear to lend some support to this hypothesis If the degree of desquamation is relatively constant increased diuresis will lead to lower white cell concentration and determinations of excretion rates might therefore theoretically give constant values

In accordance with the findings of Osborn and Smith (15) we found a close relationship between the excretion rates and the cells counted in the haemocytometer (Figs 6 and 7) The relation be tween rate of urine flow and excretion rate was loose (erythrocytes $r = 0\ 2431$ leucocytes $r = 0\ 2834$) A significant correlation between the rate of urine flow and the excretion rate of renal tubular and red blood cells but not leucocytes has been reported by Prescott (17) Pears and Houghton (16) found no correlation between volume and the excretion rate of white cells

The excretion rate is the product of cell con centration and volume In a series of specimens from various persons the number of cells per unit volume may show any value from 0 to large numbers while the volume shows only minor variations under standardized conditions The present study revealed that the volumes excreted per hour in /₃ of the specimens ranged from 20 to 49 ml with a median of 40 ml The demon strated correlations or lack of correlations be tween excretion rates cell concentrations and rates of urine flow may therefore mainly be a result of arithmetic (a variable factor being multiplied by a relative constant factor)

White cells when present in low numbers in urine are distributed in the haemocytometer in accordance with the Poisson distribution the variability of random samples following the same distribution (6) In a single sample the variability

Table II *Theoretical range of the excretion rates of 200 000 cells/h of 400 000 cells/h based on cell counts in 1 mm³ uncentrifuged urine at various rates of urine flow*

Cell concentration		Range of cell excretion rate			
Cells mm³	95 conf lim of a single count	Urine flow (ml/h)	200 000 cells/h	Urine flow (ml/h)	400 000 cells/h
1	0– 5 6	,00	0–1 1 0 000	400	0–. 40 000
2	0 2– 7 2	100	20 000– 7.0 000	.00	40 000–1 440 000
4	1 1–10 2	50	55 000– 510 000	100	110 000–1 0 0 000
5	1 6–11 7	40	64 000– 468 000	80	128 000– 936 000
8	3 5–15 8	25	87 500– 395 000	50	175 000– 790 000
10	4 8–18 4	20	96 000– 368 000	40	197 000– 736 000
20	12 2–30 9	10	1 2,000– 309 000	20	244 000– 618 000
40	.8 6–54 5			10	286 000– 545 000

of the excretion rate is therefore determined by the variability of haemocytometer counts The upper limit of the normal excretion rate per hour of white cells has b en reported to be 2–400 000 (14 15) These values may be obtained by multiplication of various cell counts by various volumes When the 95 confidence limits (4) are applied to counts in 1 mm³ of uncentrifuged urines and the excretion rates are calculated on the basis of the percentile points (Table II) the random error becomes large and may in actual practice become even larger when the observer error is accounted for (6)

In patients with chronic pyelonephritis increases of more than 100% of the excretion of leucocytes in urine have been reported after intravenous injection of lipopolysaccharides (11 14) Table II shows that in individual cases a considerable increase in the excretion rate may be accounted for by the random variability of haemocytometer counts

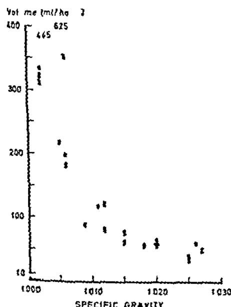


Fig 8 Rate of urine flow compared with the specific gravity of urine in 105 persons (without fluid restrictions)

Patients with urinary tract diseases sometimes have a high diuresis which might influence the excretion rate to a greater extent A study of the relationship b tween the specific gravity and the volume excreted per hour (based on 3 hour collecting period) by 105 patients without fluid restrictions revealed a considerable increase in volumes only at specific gravities lower than 1 012–1 010 (Fig 8) at which hypotonic haemolysis of erythrocytes might occur

The present study indicated that the determination of excretion rates of cells in urine under standardized conditions is of limited value in healthy males and probably has no justification The cell content in urine should be expressed in terms of cells per unit volume and in the volume actually examined the magnitude of the standard error of the estimate expressed by the confidence limits being approximately known (6) In routine work examination of 1 mm³ urine usually is most suitable

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SERUM ELECTROLYTE CHANGES DURING LONG TERM TREATMENT WITH AMILORIDE HYDROCHLORIDE (MK-870)

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Abstract Twenty two patients with severe heart disease treated with sulfamyl diuretics were given amiloride hydrochloride (MK 870) over periods of six weeks to 14 months. The potassium sparing effect of MK 870 was sufficient in all but one patient No dangerous hyper kalemia was noted There was a transitory decrease in the mean serum sodium concentration The initial high mean serum CO_2 level was decreased during the first six weeks but not during prolonged treatment The initial low mean chloride level was increased The increase was statistically significant after six weeks but not during prolonged treatment Rises in serum creatinine levels were noted in two patients, one of whom had decreased renal function but the mean creatinine level was not significantly altered The drug had no consistent effect on the serum uric acid level

The association of stenosing ulcers of the small intestine with potassium chloride tablet therapy and other disadvantages connected with oral potassium supplements have aroused increasing interest in potassium sparing diuretic agents

Amiloride hydrochloride (N amidino-3 5-diamino-6-chloro pyrazinamide hydrochloride dihydrate) has as triamterene potassium sparing properties without being an aldosterone antagonist Maximum activity of amiloride hydrochloride (MK 870) occurs 2–8 hours after ingestion of a single dose but a significant effect on both sodium excretion and potassium retention could still be seen after 24 hours (17)

In short term treatment of fluid retention (6) the drug produced a consistent rise in the serum level of potassium There was a slight decrease in the serum levels of sodium and CO_2 The agent had moderate diuretic and natriuretic activity while the potassium excretion was either decreased or not affected The median value of the sodium/potassium quotient on the day of maximal potassium retention was 9 6 as compared with 1 1 before MK 870 treatment There was also a moderate increase in the urinary sodium/chloride quotient In short term experiments the drug has been shown to increase bicarbonate excretion (10 17)

The main aim of this study was to evaluate in patients on sulfamyl diuretics the long term action of MK 870 on serum electrolytes and serum levels of creatinine and uric acid

MATERIAL AND METHODS

Twenty two patients with advanced heart disease were studied (Table I) All were in function groups III–IV according to the criteria of New York Heart Association All patients but one (case 17) were digitalized and on sulfamyl diuretics and potassium chloride supplements before the study About half of the patients had overt fluid retention when the MK 870 treatment was started

The patients were hospitalized during the first one to two weeks of MK-870 treatment They took a normal diet and fluid intake was unrestricted Digitalis and sulfamyl diuretic treatment were maintained during the study Potassium supplement administration was stopped before MK 870 was started and no patient was given potassium chloride during MK 870 treatment

The dose of MK-870 in the first week was 5 mg four times daily and then 5 mg two to three times daily All patients were treated for at least six weeks and 16 patients were treated for periods of 4–14 months

The serum levels of potassium sodium chloride carbon dioxide and uric acid were determined before MK 870 treatment was started and one week and six weeks after the beginning of MK 870 treatment During prolonged treatment blood samples for analysis were usually taken every other month

The analyses were performed by standard methods with a Technicon Auto-Analyser

Table I *Clinical data for 22 patients treated with MK-870*

Case	Sex	Age	Diagnosis	Function group	Heart size (ml/m² BSA)	Other diuretics (mg/day)	Weight loss (kg/no of days of treatment)
1	♂	61	Chronic rheumatic valvular disease Induced hypothyreosis	III-IV	870	Frmd 80-120	3 5/7
2	♀	45	Chronic rheumatic valvular disease	III-IV	540	Frmd 40-120	−1 5/14
3	♀	63	Chronic rheumatic valvular disease Diabetes mellitus	III IV	1050	Frmd 80-120	9 0/14
4	♀	64	Chronic pericarditis	IV	780	Chlth 50-100	5 4/7
5	♀	54	Chronic rheumatic valvular disease Induced hypothyreosis	III-IV	1000	Frmd 40-80	2 9/8
6	♀	57	Chronic rheumatic valvular disease Induced hypothyreosis	III-IV	850	Chlth 100 Acmd 250	2 8/6
7	o	71	Ischemic heart disease	III-IV	1070	Frmd 80	0 9/5
8	♀	67	Chronic rheumatic valvular disease	III-IV	830	Frmd 80 alt Chlth 100	0 6/7
9	♀	61	Chronic rheumatic valvular disease Diabetes mellitus	III	610	Chlth 50	2 0/7
10	♂	50	Chronic rheumatic valvular disease	III-IV	970	Frmd 80	2 3/7
11	♂	63	Chronic rheumatic valvular disease	III-IV	940	Frmd 80 alt Chlth 50	−0 5/7
12	o	20	Ventricular septal defect	III-IV	690	Frmd 40	3 0/7
13	♀	59	Chronic rheumatic valvular disease	III-IV	570	Frmd 80 alt Chlth 50	0/7
14	♀	71	Chronic rheumatic valvular disease	III-IV	960	Frmd 80	1 6/7
15	♀	59	Chronic rheumatic valvular disease	III-IV	900	Frmd 40 80	2 0/7
16	o	62	Ischemic heart disease	IV	540	Chlth 50-100	2 5/5
17	♀	45	Chronic rheumatic valvular disease	III	360	Frmd 80	0/7
18	♀	44	Chronic rheumatic valvular disease	III IV	690	Frmd 80	5 5/7
19	♀	62	Chronic rheumatic valvular disease Diabetes mellitus	III-IV	490	Frmd 40-80	1 8/7
20	♂	54	Chronic rheumatic valvular disease	III-IV	450	Chlth 50 100 alt Frmd 80	−0 3/7
21	♂	62	Chronic rheumatic valvular disease	III-IV	960	Frmd 80	4 6/30
22	o	50	Ischemic heart disease	III	560	Chlth 100	0 5/4

Chlth = chlorthalidone Frmd = Frusemide Acmd = Acetazolamide

RESULTS

The combined treatment was associated with weight loss (Table I) in most cases and after one to two weeks of treatment no patient had obvious peripheral edema This result was probably not entirely due to the addition of MK 870 because the weight of several patients was diminishing before MK 870 treatment Furthermore in some patients the dose of sulfamyl diuretics had recently been increased

As regards the influence of MK 870 on serum potassium (Table II) hypokalemia (< 3 6 mEq/l) was present in 8/22 patients before treatment with MK 870 One week after the start of MK 870 treatment hypokalemia was noted in two patients In one of them (case 6) who was on chlorthalidone (100 mg/day) earlier treatment with potassium chloride supplement of 4 g/day had not prevented hypokalemia Before MK 870 was started the serum potassium was 2 6 mEq/l and after one week of MK 870 treatment her potassium value was 3 3 She was treated for seven months and was the only patient who had

Table II *Serum potassium (mEq/l) before during and after treatment with MK-870*

	Mean ± s e	Mean diff ± s e	Signif
Before MK-870	3 8±0 14		
During MK-870		0 8±0 14 (19)	S
1 week	4 6±0 13		
		0 5±0 13 (21)	S
6 weeks	4 4±0 1[illegible]		
		0 1±0 10 (16)	N S
4-14 months	4 3±0 11		
		0 5±0 18 (10)	S
After MK-870	3 8±0 23		

[a] No of patients within brackets

Table III *Serum sodium (mEq/l) before during and after MK-870 treatment*

	Mean ± S E	Mean diff ± S E	Signif
Before MK-870	140 ± 0 4		
During MK-870		4 2 ± 0 7 (18)	S
1 week	136 ± 0 7		
		0 6 ± 0 7 (20)	N S
6 weeks	140 ± 0 8		
		0 4 ± 1 1 (15)	N S
4–14 months	*141 ± 0 8*		
		1 8 ± 0 8 (10)	N S
After MK-870	143 ± 1 2		

No of patients within brackets

Table V *Serum carbon dioxide (mEq/l) before during and after MK-870 treatment*

	Mean ± S E	Mean diff ± S E	Signif
Before MK-870	30 ± 0 9		
During MK-870		2 6 ± 0 7 (18)	S
1 week	27 ± 0 6		
		2 5 ± 0 7 (20)	S
6 weeks	27 ± 0 6		
		1 8 ± 0 8 (15)	N S
4–14 months	*29 ± 1 0*		
		0 7 ± 1 0 (14)	N S
After MK-870	29 ± 1 0		

No of patients within brackets

hypokalemia on prolonged treatment (mean value 3 1) The other patient (case 22) with hypokalemia after one week (3 3 mEq/l) had the same value initially He had no hypokalemia on prolonged treatment (mean value 4 2) One patient (case 16) had a hypokalemic value (3 1) after treatment lasting one month but not during prolonged treatment The mean potassium value after one week was 0 8 mEq higher than before MK 870 treatment ($p < 0\ 001$) Hyperkalemia ($> 5\ 1$) was noted in two patients both of whom had values of 5 4 mEq At six weeks the mean value was 4 4 and none had hyperkalemia After 4–14 months of MK 870 treatment the mean value was 4 3 This was not statistically different from the value obtained after 6 weeks treatment After withdrawal of MK 870 ten patients had a mean value of 3 8 and this was significantly lower than the mean value obtained before stopping MK 870 treatment Thus in this series of patients with advanced heart disease MK 870 failed to maintain normal serum potassium level in long term treatment in only one patient and serious hyperkalemia was not encountered

Serum sodium (Table III) was within the normal range before MK 870 treatment After one week the mean value was slightly but significantly lower than initially and hyponatremia (less than 139 mEq/l) was noted in 11/18 investigated patients After six weeks and after 4–14 months the mean values were not different from that obtained initially but hyponatremia was noted in occasional cases (lowest value 135 mEq) Two weeks after the withdrawal of MK 870 ten patients had a slightly (but not significantly) higher mean value than before stopping MK 870 treatment

The serum chloride concentration (Table IV) was low before MK 870 treatment and the mean valu was unchanged after one week After six weeks the mean value was significantly higher After prolonged treatment it was maintained on a higher level than initially but not significantly so

Table IV *Serum chloride (mEq/l) before during and after MK-870 treatment*

	Mean ± S E	Mean diff ± S E	Signif
Before MK-870	96 ± 1 3		
During MK-870		0 8 ± 1 7 (18)	N S
1 week	95 ± 1 0		
		4 0 ± 1 7 (20)	S
6 weeks	100 ± 1 2		
		1 7 ± 1 4 (15)	N S
4–14 months	98 ± 1 2		

No of patients within brackets

Table VI *Serum uric acid and serum creatinine (mg/100 ml) before and during MK-870 treatment*

	Before MK-870	During MK-870 One week	During MK-870 Six weeks
Uric acid Mean ± S E	7 2 ± 0 6 (16)	6 8 ± 0 6 (10)	6 7 ± 0 6 (16)
Creatinine Mean ± S E	1 0 ± 0 1 (21)	1 0 ± 0 1 (14)	1 0 ± 0 1 (1)

No of patients within brackets.

The carbon dioxide concentration (Table V) was high before MK 870 treatment After one week the mean value was significantly lower and remained at that level during six weeks After prolonged treatment however the mean value was not significantly lower than the initial one Before treatment a rai ed carbon dioxide concen tration (> 31 mEq/l) was noted in six out of 20 examined patients after prolonged treatment in three out of 15 examined patients

The mean uric acid level (Table VI) was some what lower but not significantly so during MK 870 treatment than before The greatest incre ment after one week was 1 3 mg/100 ml and the greatest decrement was 2 9 mg/100 ml After six weeks the corresponding figures were 1 8 and 3 5 respectively The mean serum creatinine level (Table VI) was unchanged during MK 870 treat ment In one patient the serum creatinine value rose from 1 1 to 1 9 mg/100 ml after one week After six weeks the creatinine value reverted to the initial level in spite of continued MK 870 treatment One patient (case 7) had an initial value of 2 0 This was unchanged after one week but rose to 2 9 after six weeks He later died of pulmonary embolism and anuria (see below)

Side effects

One patient (case 21) had a transitory confusion probably caused by cardial decompensation and excessive salt and water loss Two patients died during treatment with MK 870 One of them (case 5) was a 54 year-old woman who had mitral and aortic valvular disease At heart catheteriza tion in October 1966 she was found to have a severe pulmonary hypertension and a very low minute volume She had atrial fibrillation and a heart size of more than 1000 ml/m BSA In Novemb r 1965 (before MK 870 treatment) she probably had pulmonary embolism The last months she was severely disabled and mostly bed ridden She had a radioiodine induced hypothyre osis MK 870 treatment was started in the middle of October 1966 and was associated with a transi tory improvement On the 19th of February 1967 she had thoracic pain and increasing dyspnea and died on the following day At autopsy thrombotic material was found in the left auricle In two small branches of the pulmonary artery emboli were found Two cerebral malacias were found in the right hemisphere

The other patient (case 7) was a 71 year-old man who had ischemic heart disease He was ad mitted in October 1966 because of severe heart decomp nsation in spite of digitalis and sulfamyl diuretic treatment He had atrial fibrillation and a heart size of more than 1000 ml/m BSA

MK 870 treatment was started in the middle of October 1966 and was associated with a small weight loss He was admitted again at the be ginning of December because of chest pain and dyspnea He was treated with frusemide 80 mg/day MK 870 15 mg/day and acetyldigitoxine He had no edema but was tired and confused The serum electrolytes were normal He had raised serum creatinine already before MK 870 treatment On the 6th of December the MK 870 treatment was stopped He died on the 12th of December He had small urine volumes when he was admitted and on the last two days had anuria The B P was below 100 mm Hg durin the last days At autopsy parietal thrombi were found in the left ventricle of the very large heart In the pulmonary artery the right main stem was obliterated by embolic material Advanced ne phrosclero is was found In the right ureter there was a concrement the size of a pea Chemical analysis showed phosphatic stone There was cerebral atherosclerosis

It is not possible to decide whether the lethal outcome of these two patients had any causal relationship with the diuretic treatment They were both severely disabled and the course of their diseases was not surprising

DISCUSSION

From the clinical point of view the most im portant of the electrolyte disturbances provoked by sulfamyl diuretics is hypokalemia which especially in digitalis treated patients may have dangerous consequences

Hypokalemia is often induced by sulfamyl diuretics in spite of oral potassium supplements Furthermore oral potassium chloride may pro-voke serious gastrointestinal disturbances (9)

Spironolactone (11 12) or triamterene (3 8 13 16) does not always prevent sulfamyl diuretics from inducing hypokalemia

According to the literature MK 870 has pre vented hypokalemia in sulfamyl-diuretic treated patients in short term trials but there is no avail

able data as to long term treatment with the exception of one patient reported by Singh et al (15) The patient was treated for eight months with maintained potassium sparing effect In this study MK 870 was given for at least six weeks in 22 patients and for periods of 4–14 months in 16 patients The drug had sufficient potassium sparing effect in all but one patient This patient had hypokalemia before treatment in spite of a potassium chloride supplement of 4 g/day She had however a higher level of serum potassium during MK 870 treatment than before

Hyp rkalemia of dangerous levels during triamterene has been reported (5) As regards MK 870 this has been reported (15) in one patient with s vere renal failure The value in this patient increased from 6 1 to 7 0 mEq/l

In the present study slight hyperkalemia was noted in two patients after one week of MK 870 treatment (maximum value noted was 5 4 mEq/l) but not with prolonged treatment

As this series comprised patients with very advanced heart diseases it seems probable that MK 870 treatment will prove to achieve potassium balance in most sulfamyl treated patients with fluid retention of cardiac origin Whether this is true also of fluid retention of hepatic or renal origin remains to be studied

As regards other electrolytes the mean serum sodium value was significantly lower one week after starting the MK 870 treatment but then reverted to the original level The sulfamyl diuretic induced hypochloremia was reduced after six weeks During prolonged treatment the mean chloride level was higher than initially but not significantly so *The sulfamyl-diuretic provoked* alkalosis could be abolished only for a short time with MK 870

Triamterene treatment especially in daily doses above 100 mg/day is accompanied by reduced cr atinine clearance and raised serum creatinine (1 2 5 7) By contrast studies on creatinine clearance after the administration of MK 870 have shown unchanged or increased creatinine clearance (15 17) On the other hand Gombos et al (4) reported an increased serum creatinine level during hydrochlorothiazide treatment and this higher level was maintained when hydrochlorothiazide was replaced by MK 870

In the present series the mean serum creatinine level during treatment was not statistically different from that before MK 870 treatment A raised creatinine level was however noted in two patients The increment was transitory in one The other patient later died and was found to have advanced nephrosclerosis

The MK 870 treatment had no significant influence on the serum uric acid level

Two patients with advanced heart disease died It is not possible to decide whether the lethal outcome had any causal relation to the diuretic treatment

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THE RELATIONSHIP BETWEEN PLASMA RENIN ACTIVITY AND HEMOCONCENTRATION

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Abst act The relationship between renin activity and hemoconcentration determined by colloid o motic pressure has been stud ed during changes in posture and acute stud es on subjects given a diureti

1 Renin activity increased on an average three times from supine to standing position There is no d fferen e between the values after 0 and 40 minutes of standing and the values obtained after 4–5 hours of quiet walking The in rease takes place between the 10th and 0th minute after standing position has been assumed

COP increase precedes the increase in renin activity In the first five minutes 60 of the maximum increase has been attained A plateau is reached after .0 minutes of stand ng 3 A close l near relationship between Δ COP and Δ renin activity is demonstrated

Two possibilities of a causal relationship arise (a) renin a tivity increa e is a function of blood volume loss in these conditions concomitant with hemoconcen tration (b) renin activity is a function of hemoconcentra tion in these experiments expressed by COP

As post hemorrhage blood volume loss does not st m ulate an increase in renin activity the second possibility is favoured

An influence of postural pooling on the rise in renin act vity can be ruled out as a rise of the same order in COP and in plasma renin activity are found in the fu osem de studies where no pooling occurs

Chan es in renin activity due to postural changes have been described by several investigators (2 6 11) and a relationship between the changes in plasma renin and changes in blood volume has been considered (2) It has been the aim of the present investigation to study the possible rela tionship between increase in plasma renin activity due to changes in posture and postural changes in hemoconcentration determined by the variation of colloid osmotic pressure The velocity of the postural increase in renin activity and hemocon centration is examined and chang s in these two values are further studied in acute studies on subjects given a diuretic (furosemide)

MATERIAL AND METHODS

The material comprises 11 females and .1 males aged 18–58 The individuals were healthy medical students and patients hospitalized for minor conditions They had never shown any signs of cardiovascular d sease hyper tension or kidney disease and they received no dru_s All the subjects had been on a normal diet prior to the ex periment

An indwelling needle was placed in an antecubital ve n coagulation in the needle was prevented by flushing it with small amounts of 3 8 Na citrate Immediately before blood samples were obtained 3 ml of blood was drawn and discarded because of the content of Na citrate from the needle Subsequently blood samples were drawn into three disposable syringe of 10 ml each containing 1 ml of 3 8 Na citrate The blood was transferred to a siliconized 50 ml Erlenmeyer flask immersed into ice water Within one hour the blood was centrifuged at 0 C 3000 rpm for 15 minutes The plasma was separated and kept at – 20 C until renin activity was determined

The renin activity was determined by the method of *Boucher et al (1) with the mod fications we have de* scribed (coefficient of variation ± 11 8) (10) The re sults are expressed as n_ angiotens n/10 ml of plasma/4 hours of incubation In addition to the blood ob tained for measurement of renin activity another 10 ml of blood was drawn into a dry syringe for determination of colloid osmotic pressure (COP) plasma sodium plasma potassium and plasma chloride COP was re corded in an electronic osmometer for qui k direct *measurement of small samples described* by Hans n (8) The results are expressed in cm H O Confidence limits 95 = ± 0 5 mm Hg

Fawcett et al (7) have shown that healthy indi iduals do not lose significant quantities of plasma protein dur ing hemoconcentrat on to a s milar extent as reported by us. The ratio between albumin and globul n also remains unchanged as shown by Youmans et al (1)

Consequently COP is an acceptable measure for the *degree of hemoconcentration*

Plasma sodium and potassium were determined by flamephotometry chloride by potentiometric Ag^+ titra tion Standard bicarbonate was determined by a method descr bed by Jørgensen et al (9)

A Postu al studies

Eleven individuals in 12 experiments (three females eight males) All experiments were carried out between 8 a m and 11 a m

1 Seven subjects were studied as follows When the subject had been in a supine position for 45 minutes the first blood sample was obtained the subject was then assisted to a standing position To obtain a "normal" standing position the subject was *permitted to take a* few steps occasionally Blood samples were drawn 5 10 0 and 40 minutes after the subject had assumed the standing position

2 Five subjects were studied as follows The re cumbent and standing position procedure was carried out as in the group mentioned above After .0 minutes of quiet standing the subject was placed in a sitting position for 20 minutes Blood samples were obtained 5 10 and ?0 minutes after the subject had assumed a standing position and 20 minutes after a sitting position had been assumed No determination of renin activity was carried out in the blood samples drawn after 5 and 10 minutes of standing

B Furosemide studies

Six recumbent individuals in six experiments (six males) The experiments were carried out between 8 a m and 2 p m When the subject had been in a supine position for 45 minutes the first blood sample was drawn At the same time 80 mg of furosemide was given perorally together with approximately 50 ml of water From then on samples were drawn at the following intervals indi cated in relation to the time when furosemide was given 20–40–60–100 160 and 190 minutes In two cases a sample was drawn after another 30 minutes The ex perimental period in this way amounted to 3–4 hours If possible the subject urinated before the first blood sample was obtained and thereafter as often as possible

C Stud es on cont ol individuals

(Eight females and seven males)

1 Four subjects were studied as described above in section B except that they were not given furosemide

? Three subjects were studied as follows After 30 minutes of supine position 18 ml of blood were drawn for renin determination In 5–10 minutes 435 ml of blood were drawn using the other antecubital vein. In one case another blood sample for renin determination was obtained *immediately after venesectio in the two* other cases 15 minutes later

3 Eight subjects studied as follows Blood samples were drawn from an antecubital vein for determination of *renin activity after ambulation for 4–5 hours*

RESULTS

A Postural studies

1 Blood samples drawn from seven subjects for determination of renin activity and COP after 45 minutes of recumbency and 5 10 20 and 40 minutes of standing (Table I Fig 1)

Table I shows that the range of renin activity as well as COP is considerable for all the samples No relationship is demonstrated between the levels of renin activity and COP After the head-over feet position has been assumed COP increases rapidly After 5 minutes the mean rise in COP amounts to 60% of the increase after 40 minutes of standing after 10 minutes 83% and after 20 minutes 114

Thus the differences in COP from the 20-minute to the 40 minute samples are minor In

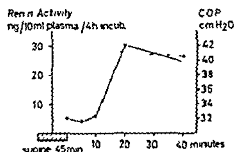


Fig 1 Variations in plasma renin activity and COP in representative case G after 5 10 ?0 and 40 minutes of standing. Renin activity is expressed as ng angiotensin 10 ml plasma 4 h incubation

Table I *Values of renin activity and COP in seven subjects after 5 10 20 and 40 min of standing*

Subj	45 min supine		5 min standing		10 min standing		0 min stand ng		40 m n stand ng	
	Renin	COP	Renin	COP	Renin	COP	Renin	COP	Renin	COP
D	31	36 6	24	38 5	[illegible]6	40 3	51	41 1	44	40 0
G	5	35 3	4	41 7	6	41 5	30	41 0	[illegible]5	40 6
H	4	36 1	6	38 1	6	40 1	13	44 3	19	42.7
K	15	35 2	16	38 1	6	41 5	37	45 8	48	44 5
L	16	35 0	14	38 5	26	37 8	[illegible]5	38 7	29	38 4
M	4	31 7	7	34 0	4	34 0	15	36 2	13	35 0
N	21	36 9	17	44 4	22	45 9	52	47 5	56	47 8

Table II *Values of renin activity in five subjects after 20 min of sitting and corresponding values of COP after 5 10 and 20 min of standing and 20 min of sitting*

	45 min supine		5 min standing		10 min standing		20 min standing		20 min sitting	
Subj	Renin	COP	Renin	COP	Renin	COP	Renin	COP	Renin	COP
A	8	30 1	—	33 1	—	37 0	24	36 8	17	33 8
B	3	35 3	—	38 7	—	39 5	16	39 1	5	37 6
C	16	31 0	—	36 7	—	35 5	32	36 9	21	33 6
E	6	32 2	—	35 0	—	36 4	27	39 7	21	35 6
F	0	36 6	—	42 4	—	44 6	24	44 2	19	41 7

the four cases where the 95 % confidence limit is exceeded the difference signifies a fall in COP

In five subjects renin activity shows no increase at all in the 5 and 10 minute samples while in two subjects no increase is demonstrated after 5 minutes in standing position but a rise exceeding the 95 confidence limit of renin activity determination is shown in the 10-minute sample

In the 20 minute sample all seven subjects showed an increase in renin activity amounting to three times the initial mean value Differences in renin activity in the 20 minute and 40-minute samples are minor and do not in any case exceed the 95 confidence limit of the renin activity determination

2 Five subjects sitting for 20 minutes after 20 minutes of standing (Table II)

The figures for the individual subjects show that while the decrease in COP in every case

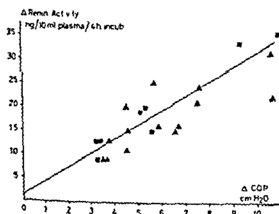


Fig 2 Correlation between increase in renin activity and increase in COP from resting values to values after 20 and 40 minutes of standing and to sitting values. Supine→sitting (●) Supine→20 minutes of standing (▲) Supine→40 minutes of standing (■) Renin activity is expressed as ng angiotensin/10 ml plasma/4 h incubation

exceeds the 95 confidence limit the decreases in renin activity with one exception (B) are borderline values in relation to the 95 confidence limit Compared to the values of renin activity in the supine position the value after 20 minutes sitting clearly exceeds the 95 % confidence limit

The relationship between Δ renin activity and Δ COP from supine position to 20 minutes of sitting and to 20 and 40 minutes of standing are shown in Fig 2 The correlation may be described as linear with the regression line $y = 2.8x + 1.4$ $r = 0.87$ ($p < 0.001$) S D of the slope is ± 12 The ordinate of the y axis intersection of the regression line is not significantly different from zero ($0.50 < p < 0.60$)

In the postural studies no changes in plasma sodium potassium or chloride were observed

B *Furosemide studies*

Six subjects given 80 mg furosemide perorally studied in supine position

In five cases the effect of furosemide as shown by increase in diuresis COP and renin activity started in the second hour of the experiment In one case it began in the first hour

In five subjects a plateau for renin activity and COP was attained prior to the termination of the experiment and in two of these an incipient decrease towards the initial level as well Fig 3 depicts the variations in renin activity and COP throughout the study in two representative cases (A and B) It shows that renin activity and COP go together to a great extent

The mean initial value of renin activity was 7 ng ± 4 (S D) range 0–12 ng The renin activity at the plateau (in the five cases attaining this) was 30 ng ± 7 (S D) range 20–40 ng The values for COP were respectively 34 5 cm $H_2O \pm 3.0$ (S D) range 30 0–38 4 cm H_2O and 41 5 cm

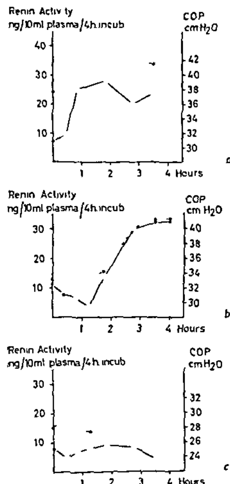


Fig 3 Variations in renin activity and COP in two cases (*a* and *b*) given furosemide and in one control case (*c*) Renin activity is expressed as ng angiotensin/10 ml plasma 4 h incubation

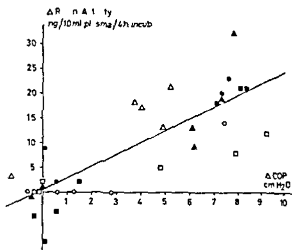


Fig 4 Correlation between Δ renin activity and Δ COP *from values after 45 minutes of supine position and all* following samples The six signatures refer to the six different individuals Notice that the deviation from the calculated line of correlation are due mainly to individual variations Renin activity is expressed as ng angiotensin/10 ml plasma/4 h incubation

H O ± 1 9 (S D) range 39 2–43 8 cm H O Both increases are significant ($p < 0\ 001$) The relationship between Δ renin activity and Δ COP from the initial values to all following values are shown in Fig 4 The correlation may be described as linear with the regression line $y = 2\ 3x + 0\ 4$ $r = 0\ 82$ ($p < 0\ 001$) S D of the slope is 12 % The ordinate of the y axis intersection of the regression line is not significantly different from zero ($0\ 30 < p < 0\ 40$) The slopes of the two regression lines do not differ significantly ($0\ 30 < p < 0\ 40$) (Figs 2 and 4)

In these studies no changes in plasma sodium potassium chloride or standard bicarbonate were observed

C *Control studies*

1 The four experiments corresponding to the furosemide studies show no increase in renin activity or COP during the experiments A representative study is shown in Fig 3 *c* No chan es were observed as to plasma sodium potassium chloride or standard bicarbonate

2 The experiments in which 435 ml blood were drawn during 5 minutes did not show an increase in renin activity in any of the cases Comparing the blood samples before and after the venesectio no signs of hemodilution were observed

3 Eight individuals in erect posture for 4–5 hours Mean value of renin activity *26 ng ± 8* (S D) range 17–42 ng This mean value is not significantly different from the m an values obtained after 20 and 40 minutes of standing ($0\ 2 < p < 0\ 25$)

DISCUSSION

The present study on the whole confirms the findings of other investigators as regards pos renin increase (2 6 11) An increase in renin activity is found after 20 minutes of which is approximately three times the value at rest No additional increase is

strated after 40 minutes of standing The means of these two values of increase in renin activity do not deviate from renin activity determined in ei ht other healthy subjects after 4–5 hours of erect position

The rise in renin activity takes place between the 10th and 20th minute after standing position has been assumed As the mean decline in plasma volume amounts maximally to 16% correspond ing to a rise in renin activity of 300% the in crease in renin activity cannot be explained by a simple concentration of plasma proteins That the rise is due to postural changes and not to diurnal variations is illustrated in the four control subjects who showed no rise in renin activity from 9 a m to 2 p m At the time when renin activity initially increases the COP has almost reached a plateau (Fig 1) Matched with the close correlation between Δ COP and Δ renin activity two major possibilities for a causal rela tionship to renin increase appear

1 Renin increase is a function of blood volume loss in these conditions concomitant with hemoconcentration

2 Renin increase is a function of hemocon centration in these experiments expressed by COP

It is not very likely that the postural reduction of blood volume is the provoking factor in the rise of renin activity as the drawing of 435 ml of blood does not result in increased renin ac tivity This has been demonstrated by Brown et al (4) and in this study as well

The blood volume loss from supine to sitting position is approximately of similar magnitude and in these experiments a clear-cut rise in renin activity was observed The possibility remains however that the loss of blood volume in the two situations (sitting position and venesectio) is not quite comparable due to the pooling of blood in the lower extremities in the sitting position

The importance of the pooling phenomenon seems to be ruled out when we consider the furosemide studies As the subjects are constantly supine no pooling will take place during the course of the experiment yet in these experi ments a rise in renin activity and COP of the same order as in the postural experiments is established (Figs 2 and 4) In contrast to the postural studies no delay in renin activity in rela tion to hemoconcentration has been observed in the furosemide experiments This may however be due to longer intervals between blood samp lings in this group

Brown et al (5) have shown that it was possible completely to inhibit the renin increase in dogs given chlorotiazide when saline was infused in travenously during diuresis in volumes corre sponding to the diuresis Accordingly these in vestigators suggest that renin release is stimulated by volume depletion It must be stated that in these experiments no distinction can be made be tween the effect of volume per se and hemocon centration While changes in renin activity in postural and furosemide studies reflect changes in hemoconcentration plasma sodium remains un changed In certain pathological conditions (hy pertension cardiac failure Addison s disease and salt losing kidney) Brown et al (3) have de monstrated a close inverse linear correlation be tween plasma sodium and renin activity and sug gested that decrease in plasma sodium concentra tion stimulates renin secretion We have shown the same correlation in the studies of chronic sodium depletion in healthy subjects (10)

It must be borne in mind that the relationship between plasma sodium and renin activity has only been demonstrated in chronic situations and experiments

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FAMILIAL PLASMA CHOLESTEROL ESTER DEFICIENCY

Clinical Studies of a Family

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Abstract Clinical and biochemical studies of three adult sisters with plasma cholesterol ester deficiency revealed that this syndrome is characterized by nearly complete deficiency of plasma cholesterol ester and α lipoprotein associated with marked corneal opacities normochromic anemia, proteinuria slight elevation of serum acid phosphatase and foam cells in the bone marrow The two elder had a marked increase of total cholesterol total phospholipids and triglycerides in plasma and the eldest also an increase in serum uric acid

Clinical studies of their family showed that it enjoyed good health and most family members have reached a high age without corneal renal or anemic symptoms

Plasma lipid studies including cholesterol ester and fractionated phospholipid examinations failed to elucidate the heredity of the plasma cholesterol ester deficiency

A new familial disease characterized by nearly complete deficiency of plasma cholesterol ester and of α lipoprotein associated with anemia proteinuria and marked corneal opacities was reported in a recent clinical study by Gjone and Norum (4) of the eldest of three adult sisters with this syndrome Norum and Gjone (8) have further shown that the plasma cholesterol ester deficiency is due to a plasma esterification failure as no lecithin cholesterol acyltransferase could be demonstrated in the plasma from any of these three sisters

A clinical survey of their family has been made Detailed clinical and biochemical studies of the two younger sisters with the same plasma lipid and lipoprotein abnormalities have also been carried out. The results of these studies are the subject of this report

MATERIAL AND METHODS

Relatives

The families of both parents come from a small community with a stable population in Western Norway Interviews and old letters have made family tracing possible All great grandparents are known (Fig 1) Of the great great grandparents nine of 16 are known—six maternal and three paternal Six of the 16 maternal great great great grandparents are known. The pedigree information obtained gave no evidence of intermarriage This possibility has however not been excluded in the generations earlier than the great grandparents

Father's family

The known members in directly ascending line have enjoyed good health far into the senium Three of his aunts had diabetes mellitus in old age and two of his uncles died 40–50 years old of heart disease and cerebral hemorrhage respectively

The father and his siblings—nine altogether—were born in the period 1887–1905 The father (III 6) has always been in good health until 1963 when he had a coronary infarction and was hospitalized for some weeks with a good recovery He has very slight lipoid arcus corneae but no other corneal opacities His urine is normal and he has no exanthemas xanthomas or xanthelasmatas and no uric acid tophi His tonsils are normal The same normal findings were noted in all his siblings who were examined The eldest brother (III) died ?9 years old of tuberculosis The eldest sister (III 3) died in an accident at eight years of age and a younger brother (III 7) was drowned at the age of 1 Another brother (III 8) is mentally reduced but organically healthy 67 years old

A son of the father's sister (III 4) died 14 years old of liver disease after one year of illness

The father's first wife (III 1) died in 19_8 of tuberculosis She had no abortions or stillbirths and her four daughters in this marriage are alive and healthy

Mother's family

No known serious or inheritable diseases have occurred in this family A daughter of the mother's sister however

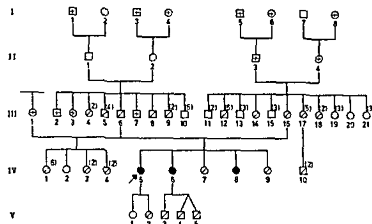


Fig 1 Family tree showing the known members of five generations The propositi are shown in solid symbols The hatched symbols indicate relatives examined and the crossed symbols those who are dead The number of children of the members of generations III and IV are given in brackets

died 17 years old of acute leukemia and a son of another sister has had an attack of podagra (IV 10 born in 1936) He has slight ear tophi but normal urine tonsils corneae and hemoglobin His serum uric acid was however 4 4 mg

The mother (III 16) has always been healthy She has given birth to five children and most probably had one or two early abortions but no stillbirths In June 1967 she had an acute fibrinous ileitis successfully treated with antibiotics She is normotensive She has normal tonsils normal urine normal hemoglobin, no uric acid tophi no arcus lipoides corneae and no xanthomatous deposits

All the ten siblings of the mother are alive and healthy Those who have been examined are all normotensive without corneal opacities or xanthomatous deposits Normal urine and normal tonsils have been found and no uric acid tophi were discovered

The siblings of the propositi

The elder of the two sisters with normal plasma lipids and lipoproteins (IV 7) has a marked oscillating nystagmus She has always been in good health but has not been pregnant during six years of marriage

The youngest sister (IV 9) is completely healthy Neither of these two has corneal opacities proteinuria or anemia

The children of the propositi

The eldest of the three propositi (IV 5) delivered her first child (V 1) in 1955 a girl born at full term 53 cm in length and 3? 0 g in weight The pregnancy was normal apart from a constant proteinuria At birth the umbilical cord was placed around the neck of the child who was cyanotic and very weak Treatment with oxygen and suction brought some improvement One day later she died however The clinical examination showed no abnormalities Autopsy was not performed

During her next pregnancy (1958) a constant proteinuria of approximately / ‰ albumin was observed B.P was normal Because of overdue pregnancy a caesarean section was performed and a girl (V 2) was born. She is healthy

Also the three sons (V 3–4–5) of the second eldest sister (IV 6) are completely healthy The twins are dizygotic

Blood samples

Fig 1 shows the family tree of the propositi The 3 members shown with hatched symbols were personally examined and twenty ml of blood from the antecubital vein was collected EDTA (1 mg/ml) was used as an anticoagulant The blood samples were cooled kept at 5 C for 1–3 days and then centrifuged The plasma was analysed for free and esterified cholesterol (9) triglycerides (6) phospholipid fractionation (5) electrophoresis of the plasma lipoproteins (7)

Plasma lipids in the family

Table I shows the results of the plasma lipid studies. It is seen that reduction in the cholesterol esterification and the lysolecithin level is present only in the three propositi A fall in the percentage of esterified cholesterol with increasing age is noted The electrophoresis of the plasma lipoproteins revealed a normal pattern

Clinical and biochemical data of the three propositi

Data from the study of the eldest sister A R (IV 5) has previously been published (4) The second eldest of the sisters—I S (IV 6) was born in 1935 Proteinuria was discovered in 1955 She has three healthy children, a 9 year-old son (V 3) and two 7 year-old twin boys (V 4-5) She was appendicectomized at the age of 14 and tonsillectomized at the age of 16 She has since childhood suffered from mild but frequent infections—common colds tonsillitis cystitis and in 1960 pyelitis Otherwise she has been healthy

The youngest of the three propositi M R (IV 8) was born in 1947 Proteinuria was discovered in 1964 and this has since been constant but moderate She had an acute tonsillitis in July 1966 with a rise of AST to 4500 She was given penicillin for two weeks Later on, however she has had recurring joint pains

A thorough inpatient examination was performed of the two youngest sisters They were both tall and slender —176 cm and 175 cm in height and 67 kg in weight.

Table I *Plasma lipid values*

No	Sex	Age	Cholesterol Total (mg/100 ml)	Cholesterol Free (mg/100 ml)	Cholesterol Esterified ( )	Triglycerides (mg/100 ml)		Total lipid phosphorus (μgP/ml)	Phospholipid fractions: Lysolecithin (μgP/ml)	Lysolecithin ( )	Sphingomyelin (μgP/ml)	Sphingomyelin ( )	Lecithin (μgP/ml)	Lecithin ( )	Cephalin (μgP/ml)	Cephalin ( )
III_4	♀	76	372	16	56 5	144	NF	127 0	6 7	5 3	22 1	17 4	92 5	72 8	5 7	4 5
III_5	♂	74	280	1 0	57 1	132	NF	89 5	5 1	5 7	12 7	14 2	67 7	75 6	4 0	4 5
III_6	♂	72	350	106	69 8	108	F	78 9	3 7	4 7	13 9	17 6	57 3	72 5	4 0	5 2
III_8	♂	67	268	102	62 0	30	NF	76 0	3 2	4 2	10 9	14 3	59 7	78 6	2 2	2 9
III_9	♂	65	280	116	67 1	104	NF	74 2	3 0	4 0	10 8	14 6	57 7	77 8	2 7	3 6
III_{12}	♂	65	252	94	62 7	100	NF	58 6	2 6	4 4	9 9	16 9	43 4	74 1	2 7	4 6
III_{14}	♀	61	334	166	50 3	244	NF	88 2	3 8	4 3	16 9	19 2	63 5	72 0	4 0	4 5
III_{16}	♀	58	290	94	67 6	141	F	95 1	4 2	4 4	18 2	19 1	69 1	72 7	3 6	3 8
III_{17}	♀	57	280	106	62 2	191	NF	73 7	7	3 7	14 2	19 3	52 8	71 6	4 0	5 4
III_{18}		56	340	136	60 0	123	NF	87 7	3 6	4 1	17 7	20 2	63 0	71 8	3 4	3 9
IV	♀	47	372	124	66 7	124	NF	95 4	5 5	5 7	18 4	19 4	68 0	71 3	3 5	3 6
IV	♀	44	360	116	67 8	108	NF	91 3	5 5	6 0	16 2	17 7	65 6	71 9	4 0	4 4
IV	♀	42	244	70	71 4	134	NF	73 0	3 5	4 8	13 0	17 8	53 3	72 9	3 2	4 5
IV [a]	♀	33	350	340	2 9	312	F	159 5	2 1	1 3	15 4	9 7	135 7	85 1	6 3	3 9
IV [a]	♀	31	580	580	0	573	F	323 8	5 9	1 8	36 6	11 3	269 5	83 3	11 8	3 6
IV	♀	25	208	54	74 1	38	F	59 3	2 8	4 7	10 1	17 1	43 8	73 8	2 6	4 4
IV_8	♀	0	143	136	4 9	129	F	83 7	1 7	2 0	9 7	11 6	69 3	82 8	3 0	3 6
IV	♀	15	244	70	71 4	126	F	63 3	2 7	4 4	10 3	16 2	47 7	75 2	2 6	4 2
IV_0	♂	31	244	80	67 3	119	NF	57 4	2 2	3 8	9 4	16 4	41 6	72 5	4 2	7 3
V_1	♀	8	148	44	70 3	78	F	55 7	2 0	3 6	6 7	12 1	44 7	80 0	2 3	4 3
V	♂	9	190	48	74 8	45	NF	47 9	2 3	4 9	6 9	14 4	37 0	77 0	1 7	3 7
V	♂	7	166	48	71 1	62	NF	56 6	2 6	4 7	9 0	16 0	42 8	75 3	2 2	4 0
V	♂	7	164	43	73 8	29	NF	49 5	1 6	3 2	7 8	15 9	37 8	76 3	2 3	4 6
Normal range			1 0–270		60–80	10–140	F	60 3–77 2		3 7–9 3		12 6–21 8		68 6–77 5		9–5 6

NF = non fasting F = fasting
[a] the propositi

Table II *Laboratory values of three sisters with plasma cholesterol ester deficiency*

	AR IV_5	IS IV_6	MR IV_8
ESR (mm/h)	20 59	31-35	17-13
Hemoglobin (g/100 ml)	8 7-10 5	9 5	10 5-11 5
Red blood cells (mill/μl)	2 9-3 7	3 88	3 58 3 47
Color index	1 02-0 96	0 95	1 12
White blood cells (/μl)	4500-7600	4100-5300	3700-4500
Platelets (μl)	113000-148000	132000-179000	143000
Reticulocytes (/1000 red cells)	1-16	18	14
Serum iron (μg/100 ml)	30-90	85	170
Transferrin (μg/100 ml)	140	240	270
Bilirubin (mg/100 ml)	1 1	0 7	1 1
Hematocrit ( )	26-33	32	36
Osmotic fragility	Normal	Normal	Normal
Haptoglobin (mg/100 ml)	Present (turbid)	135 (turbid)	85
Bleeding time (min)	9-10 5	13	9-10 5
Acid phosphatase (int units)	*9 7-14 0*	*12 0*	*13 5*
Urea (mg/100 ml)	45-65	44	30
Creatinine (mg/100 ml)	1 0-1 3	1 1	0 9
Uric acid (mg/100 ml)	8 6-10 3	4 6	3 9
Total serum protein (g/100 ml)	5 2-6 6	5 3	5 5
Serum albumin (g/100 ml)	2 3-2 9	3 1	3 1
Serum γ globulin (g/100 ml)	1 2-1 6	1 1	1 3
AST	80-120	1 0	400
B P (mm Hg)	140 90	140 80	130 80

They have marked corneal opacities as the only abnormal finding on clinical examination Their plasma lipid values have been given previously (4) and are also seen in Table I (IV 6 and IV 8) An almost complete absence of esterified cholesterol and very low relative amounts of plasma lysolecithin were found The elder (IV 6) had abnormally high values of total cholesterol triglycerides and total phospholipids whereas the youngest sister (IV 8) had normal values of total cholesterol and total phospholipids Lipoprotein-electrophoresis and immunoelectrophoretic studies demonstrated almost complete lack of α lipoprotein in plasma (8)

Table II gives some other laboratory data for the three sisters They all have proteinuria most often from traces up to 1 mg ml some microscopical hematuria but normal kidney function as judged from the urine specific gravity (1016-1020) serum creatinine and serum urea Granular casts were found occasionally in the urine of A R (IV 5) but not of the others All of them had low serum proteins with low albumin but normal γ-globulin fraction Serum uric acid was markedly elevated in the eldest (IV 5) but normal in the two others Acid phosphatase was slightly elevated in all of them but with normal tartrate titratable fraction

The hematological study showed a normochromic anemia in all of them and elevated ESR in the two elder Transferrin and serum iron were low on the first admission of A. R the two other sisters, however had normal values They all had a bone marrow with normal cellularity a slight increase in the number and immaturity of the erythroid cells but no abnormality in myelopoiesis or thrombopoiesi Some large foamy cells were seen in the bone marrow

Normal values—not listed in Table II——were found for sodium potassium, chloride calcium phosphorus alkaline phosphatase blood glucose pH pCO_2, standard bicarbonate and liver function tests (thymol SGPT PP concentration and serum bilirubin)

DISCUSSION

The clinical study of the family showed some cases of diabetes mellitus and one with arthritis urica but did not reveal any other hereditary diseases Most of the family members have reached a high age The father of the propositi is the only one who has had a coronary infarction There are no other known cases of chronic kidney or liver diseases and no case of mors subita There have been no stillbirths There are no members with chronic exanthema xanthoma or xanthelasma and no case of chronic anemia Apart from the three propositi there are no known members with marked corneal opacities As a whole therefore the large family of the propositi must be characterized as one having good health A peculiar fact is that the father has nine daughters and no son

The study of the three sisters in whom we have found almost complete absence of esterified cholesterol in plasma shows that marked corneal opacities were the only distinct clinical abnormality They have further in common deficiency of plasma α lipoprotein relative decrease of

plasma lysolecithin and increased amounts of lecithin normochromic anemia proteinuria slight elevation of serum acid phosphatases and foam cells in the bone marrow The two elder had a marked increase of total cholesterol total phospholipids and triglycerides in plasma

The present study thus shows that this plasma lipid abnormality is accompanied by characteristic abnormalities in blood and urine These features should therefore be regarded as typical of this new familial disease

It is conceivable that the normochromic anemia in all three sisters is of the same nature and due both to a slight hemolysis and to a reduced ability to compensatory increase of erythrocyte production as demonstrated in the eldest (4) A possible mechanism for this anemia may be abnormalities in the lipoprotein structure of the erythrocyte membrane The lecithin cholesterol acyltransferase reaction is probably of importance for the formation of plasma lysolecithin The lysolecithin in plasma is known to be bound to albumin and to be transported into the erythrocytes where reacylation to lecithin takes place (10) The lysolecithin is obviously a normal intermediate in the phospholipid metabolism of the red cell membrane (3) Whether a similar mechanism is present in th bone marrow during the formation and maturation of the erythroid cells is unknown but possible Alterations in the exchange of cholesterol between plasma and the erythrocytes probably regulated by the esterification reaction may theoretically change the membrane structure and characteristics The proteinuria might probably also be explained as a result of alter d membrane permeability due to changes in the lipid components of the cell membranes It does not seem conceiveable that the proteinuria can be secondary to the deposits of the possible lipid material in the foam cells seen in the kidney biopsy from the oldest patient

The increase in serum acid phosphatases may be explained as a feature of a slight hemolysis and possibly also thrombolysis since a moderate thrombopenia was present at least in one of them

Elevation of serum uric acid is seen in association with elevated serum triglycerides (2) This might explain th high serum uric acid in the eldest sister One of the others however I R with normal serum uric acid has the highest triglyceride level and the uric acid elevation is therefore difficult to explain on this basis alone which may support the view of Benedek (1) who found no such correlation

Our present study has not made it possible to elucidate the heredity of the plasma cholesterol ester d ficiency It seems reasonable that this disease is genetically determined and it is very probable that the defect is a deficiency in plasma lecithin cholesterol acyltransferase Whether the plasma α lipoprotein deficiency is secondary to this defect or there are two genetically linked defects r mains to be demonstrated The presented values for the plasma lipids have failed to reveal any carrier state for this disease Most probably however the inheritance is recessive or intermediate This will be revealed by measuring the activity of the plasma lecithin cholesterol acyltransferase the concentration of the plasma α lipoproteins and comparing them with the genetical marker systems in the family memb rs

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ANEURYSM OF THE HEART AND THE POST MYOCARDIAL INFARCTION SYNDROME

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Abst act Two fatal cases of the post myocardial infarc tion syndrome are described Autopsy revealed an aneurysm of the heart in both cases Almost all fatal cases of PMIS in the literature have had aneurysm of the heart, to which no attention has previously been paid The s gnificance of this discovery is discussed

Sporadic reports of effusive pericarditis following acute myocardial infarction are to be found in the literature before 1955 In that year Dressler de scribed a new syndrome which he called the post myocardial infarction syndrome (PMIS) also known as Dressl r s syndrome which occurred in the weeks following an acute myocardial infarc tion with prolonged or recurrent fever and pains of pleuropericardial type Symptoms in the joints have also been reported (3) Dressl*r s reports (6 7 8) have been confirmed by many others but there is no agreement as to frequency which most observers calculat to be about 1% of all myo-cardial infarctions The explanation is certainly that PMIS is confused with reinfarction pul monary embolism or pleuropneumonia and is therefore not id ntified in retrospective examina tions If the PMIS is borne in mind it will be seen that the syndrome is not unusual and that Dress ler s figures of 3–4 ⁄ are even rather low Corti sone has a rapid effect but treatment in milder cases is unnecessary On the whole PMIS is re garded as benign and there are descriptions of only a few fatal cases Autop y findings have been unspecific and have made no contribution to solving the PMIS mystery Th re is therefore sufficient justification to give a short account and dis ussion of two fatal cases of PMIS in both of which autopsy revealed a ventricular aneurysm

CASE REPORTS

Case 1

A 66-year old man with maturity onset diabetes and arterial hypertension was admitted on July 30 1967 with symptoms of acute myocardial infarction Two weeks later tachycardia and elevated sedimentation rate were noted The patient had no discomfort but on deep breathing he complained of pain in the precordium in a limited area where a pericardial friction rub could also be heard The same evening the patient developed severe chest pains and heart failure which was corrected by conventional therapy and intravenous hydrocortisone There were no symptoms of reinfarction Forty to fifty ml of san uineous fluid was drained off by pericardio centesis Haemiplegia developed and despite cortisone treatment the patient died 11 days later Autopsy re vealed pronounced nephrosclerosis with multiple cortical scars in the kidneys congestion and oedema in the lun s 100 ml of sanguineous fluid in the peri ardium severe atheromatosis in the coronary arteries and in the anterior wall of the heart an extensive 3–4-week-old in farct on with parietal thrombosis and an aneurysm in its in tial sta es A lar e encephalomalacia was found in the left occipital lobe

Case 2

A 58 year-old man with longstand ng arterial hyperten sion and a myocardial infarction in 1961 was admitted on Jan 12, 1967 with his second myocardial infarct on Four to five days after adm ssion fever and chest pains developed and these increased with deep breath ng and movement No definite per cardial friction rub was heard Card al X ray examination ten days after admis-sion re ealed effusive pericard tis and a small pleural effusion on both s des After a few days the patient im proved spontaneously During convalescence a severe venous thrombosis developed in the right calf and on Feb 4 the patient a a n developed fever and chest pains On Feb 7 when the patient was mor bund .5 mg pred nisolone was adm nistered with d amatic effect, and on the next day the patient s temperature was normal X ray e amınat on after six days cort cotherapy showed that the heart had rega ned ts normal appearance The pa t ent was d scharged after a week s prednisolone treat

ment but when he broke off the treatment at home a further flare up occurred Three months later the patient died after being taken ill with symptoms of pulmonary embolism. Autopsy revealed an extensive haemorrhagic pulmonary infarction and two older myocardial infarctions, one situated in the posterior wall and the other in the anterior wall near the apex In the latter a small ventricular aneurysm had developed over which was noted fibrous pericarditus

DISCUSSION

In the PMIS literature there are descriptions of a further nine fatal cases in which autopsy was carried out Ventricular aneurysm was established in six of these cases (1 5 8 12 16 18) Markoff (17) does not describe aneurysm in his two cases but at least one of these does not comply with Dressler's criteria for PMIS Dressler himself gives an account of two fatal cases one with (8) and one without (7) aneurysm

Of patients with non fatal PMIS some cases of ventricular aneurysm are described (2 9 11 19) *In an unexpectedly large number of cases ECG* changes characteristic of aneurysm are described although this possibility has not been discussed Levin and Bryk (15) on the other hand who X rayed more than 100 of Dressler's PMIS patients did not observe among them a single aneurysm of the heart It is however difficult to diagnose aneurysm of the heart in vivo especially small aneurysms with slight functional significance In some cases Levin and Bryk (15) found an unusual bulge of the anterior surface of the cardiac shadow on the lateral projection but they ascribe this to effusion in the pericardial sac

The connection shown between aneurysm of the heart and PMIS can hardly be coincidental Several causes may be considered

1 The severe inflammatory reaction in PMIS can be one of many predisposing factors in the development of an aneurysm

2 Cortisone treatment may possibly hinder the healing of an infarction with the development of an aneurysm as a result *Several of the cases de*scribed have been given large doses of corticosteroids for a long period before death

3 Aneurysm of the heart may have pathogenic significance The same symptomatology can be observed for instance in the post-commissurotomy or post pericardiotomy syndrome (14) and in patients with foreign bodies in the pericardium (20) Common to these two conditions is irritation or injury of the pericardium possibly with secondary haemorrhage in the pericardial sac It is not unrealistic to imagine the same mechanism in the case of PMIS An infarction which affects the walls of the heart from endocardium to epicardium could have as result (*a*) irritation of th overlying pericardium (*b*) the formation of an aneurysm The primary factor therefore would not be an aneurysm but an infarction which affects the heart tissue in contact with the visceral pericardium

The manner in which this irritation of or injury to the pericardium can give rise to the polyserositis pattern characteristic of PMIS is not known The most popular theory as to the aetiology of PMIS is that of an autoimmune mechanism It is believed that in the myocardial necrosis changes in the myocardial antigenes occur which cause the antibody building apparatus of the organism to start producing antibodies to its own myocardial tissue Reports of circulating myocardial antibodies in the PMIS have come from many sources but these discoveries are controversial as such antibodies are also present in acute myocardial infarction and coronary insufficiency with no sign of PMIS nor is there any correlation between antibody titre and PMIS (10 13) Animal experiments also argue against this theory as it has not been possible to show damage to the heart despite injections of serum with high titre of homologous myocardial antibodies (4) The presence of myocardial antibodies in patients with PMIS is presumably secondary and without pathogenic significance but before abandoning the autoimmune theory one should perhaps in these patients try to demonstrate the presence of auto-antibodies with isolated *pericardial* tissue as antigen Circulating pericardial antibodies would be a more likely explanation of inflammatory symptoms in the pleural peritoneal and synovial membranes as these membranes are ontogenetically close to the pericardium

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CHLORPROPAMIDE TREATMENT IN DIABETES INSIPIDUS

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Abstract The effect of chlorpropamide upon the polyuria of diabetes insipidus was studied in nine patients In seven patients suffering from the idiopathic type a normal or almost normal diuresis, not exceeding two liters has been obtained In the nephrogenic type chlorpropamide had no effect upon the polyuria In the polyuric phase after hypophysectomy a striking effect was seen

Hypofunction of endocrine glands is usually treated successfully by substituting the lacking hormone In some instances excellent results are achieved by oral therapy (e g thyroxine cortisone) in others parenteral therapy is necessary because of the protein nature of the hormone (e g insulin) However good results can also be obtained by using substances which are not actual hormones but are able nevertheless to correct or ameliorate the hypofunction in hypoparathyroidism vitamin D will correct the hypocalcemia and the clinical symptoms in many diabetics sulfonylureas will be of value Strangely enough some antidiabetic substances can be used in diabetes insipidus also The diguanides have some effect upon the polyuria especially in combination with a thiazide preparation (3) but chlorpropamide works even better This was reported in 1966 by Arduino et al (1) We have been able to confirm the results obtained by these authors The present paper contains our experiences from the treatment with chlorpropamide in nine cases of diabetes insipidus

CASE REPORTS AND RESULTS OF TREATMENT

Idiopathic Diabetes Insipidus

Case 1

A 21 year-old female suffered from polyuria amounting to about 10 l a day of about six years duration She had for three years been treated with intramuscular injections of vasopressin (Pitressin tannate) but as frequent injections were necessary she was admitted for evaluation On examination the diagnosis of idiopathic diabetes insipidus was confirmed both clinically and by the Hickey Hare test The results of the chlorpropamide therapy will be seen from Fig 1 During ten days observation the diuresis was 9 to 11 l Upon administration of 500 mg of chlorpropamide it decreased to about one l increased after discontinuation of the drug to 5 l and decreased after readministration to about one l a day She has later been given 375 to 250 mg of chlorpropamide daily and has had a diuresis of 1100–1 00 ml a day No side effects have been observed

Case 2

A 2? year old unmarried female had symptoms of diabetes insipidus since she was ?3 years of age On admission to the hospital a polyuria of 7700 to 1 000 ml was found and the diagnosis of diabetes insipidus was made on the clinical history and the Hickey Hare test She was given 500 mg of chlorpropamide whereupon the diuresis decreased from about 8000–10 000 ml to about 1000 ml (Fig 1) Upon discontinuation of the drug a polyuria of about 10 000 ml reappeared The polyuria was later easily controlled by daily administration of ?50 mg of chlorpropamide

Case 3

A 5? year old male the father of case ?, had from ?–3 years of age suffered from heavy water drinking, polyuria at night and disturbed sleep On admission to the hospital the diagnosis of diabetes insipidus was confirmed by the Hickey Hare test He was given 500 mg of chlorpropamide daily after a month ?50 mg and has later had a diuresis of about 1 00 ml a day (Fig 1)

Case 4

A 30-year old nurse had from childhood been drinking large amounts of water and suffered from heavy polyuria When she was ?4 years old treatment was started with pitressin injections, 5 I U every other day with good results After admission to the hospital pitressin was substituted by chlorpropamide 500 to 375 mg daily On this treatment the diuresis was about 1000 ml a day (Fig 1)

Case 5

A 49 year-old housewife noticed at the age of 44 increased thirst with a fluid intake between 8000 and 14 000 ml The diagnosis of diabetes insipidus was made

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DEPRESSION OF ISOPRENALINE INDUCED IDIOVENTRICULAR RHYTHM IN MAN BY BETA ADRENERGIC RECEPTOR BLOCKING AGENTS

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Abstract A method for studying drug effects on cardiac automaticity in man is presented

During the treatment for Stokes-Adams disease emergency resuscitation from ventricular standstill can be effected by the arousal of intrinsic ventricular pacemakers Isoprenaline seems to be a useful drug to produce idioventricular rhythm but as shown in the present investigation its effect varies widely from patient to patient.

The isoprenaline induced increase in the ventricular automaticity was depressed by the beta receptor blocking compounds propranolol and INPEA. The compounds seemed to block this effect of isoprenaline more easily than the effect of isoprenaline on the sino-atrial rate The observations indicate that adrenergic beta receptors are significantly involved in the control of cardiac automaticity in man.

The observed effects of the beta receptor blocking drugs have an important clinical implication. In patients treated with the drugs the efficiency of beta mimetic substances in emergency resuscitation from ventricular standstill may be critically reduced

The adrenergic influence on automatic rhythmic activity in cardiac muscle is not yet satisfactorily described Animal experiments have indicated that adrenaline and isoprenaline can induce idioventricular rhythm and that the response is mediated by beta adrenergic receptors (3 11) So far however no such receptor studies have been performed in man

The aim of the investigation was to study the influence of beta receptor blocking agents on the effect of isoprenaline on the ventricular automaticity in man and to try to evaluate the role of beta receptors in the control of this function.

Patients with complete atrioventricular block and intracardiac electrical stimulation were studied When the artifical pacemaker is stopped suddenly an idioventricular center may or may not restart the ventricular activity If ventricular activity is produced it starts after a variable interval of asystole (5 9 13) Changes in this spontaneous activity were studied after the administration of isoprenaline before and after treatment with the beta receptor blocking substances propranolol (AB Scanmeda Goteborg Sweden) (2) and INPEA (AB Draco Lund Sweden) (12)

METHODS

Patients with complete atrioventricular block and regular sinoatrial activity treated with an artificial pacemaker were studied All were supplied with an external pacemaker with variable frequency and voltage (Elema Schonander type 138) which stimulated a catheter electrode that had been introduced into the right ventricle through v jugularis externa (6) An indifferent electrode had been placed subcutaneously by operation.

The patients were informed about the purpose of the experiment and how it would be carried out and they all consented to it. The stimulation was broken at their usual pacemaker frequency—generally 70 impulses per min—by turning the voltage regulation to the "off" position. The interruption of stimulation lasted for 4–8 sec which the patients tolerated well If no spontaneous ventricular activity was established within this period the stimulation was started again so that a regular ventricular rhythm provoked by the pacemaker returned After 5 min continuous treatment with pacemaker an identical break was made Then isoprenaline (Isuprel) was injected intravenously in a dose of 6 8 or 1. μg in 5 5% glucose solution The drug was given in the smallest individually ascertained dose that was needed to produce idioventricular rhythm following each interruption of stimulation The effect of isoprenaline on the atrial frequency was maximal about 45 sec after the injection and was

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Table I *Age duration of pacemaker treatment heart volume blood pressure and daily drug therapy in the patients*

Patient	Age	Pacemaker treatment (y)	Heart volume (ml/m² BSA)	Blood pressure (mm Hg)	Daily drug therapy		Investigated drug
K Ö ♀	56	½	340	140 75	0		Propranolol
A E ♀	72	1½	470	150/70	Chlorthalidone	50 mg	Propranolol
					Protamine zinc		
					Insulin	24 I U	
T F ♀	73	1	400	190/110	Chlorthalidone	50 mg	Propranolol
C. C. ♂	71	5	540	150 80	Digoxin	0 ⁷5 mg	Propranolol
					Fursemide	20 mg	
					Proxiphylline		
K Ö ♀	56	½	340	140/75	0		INPEA
R J ♂	71	4	450	165/85	0		INPEA
C. L. ♀	70	2½	570	135/65	Polythiazid	0 5 mg	INPEA
G S ♀	72	3	750	125/80	Digoxin	0 ⁷ mg	INPEA
					Chlorothiazid	500 mg	
O E. ♀	82	1½	650	145/75	Digoxin	0 25 mg	
R R ♂	75	7	460	180 80	Dicumarol		
H W ♂	66	1	690	160 85	Chlorpropamide	0 25 g	
M H ♀	70	1½	480	180 95	0		
G W ♂	63	4	500	150 90	Lanatoside C	0 5 mg	
					Meprobamate		

reduced or had disappeared after 2 min The effect on the ventricular automaticity was for this reason estimated 45 sec after the injection except in one patient in whom the effect of isoprenaline on the atrial frequency and the ventricular rhythm was most pronounced 2 min after the injection A further injection of the drug in the same dose was given 5 min later to four patients.

If idioventricular rhythm had been provoked by 12 μg or less of isoprenaline the experiment was continued After the artificial pacemaker had taken over the heart rhythm once again 1 mg propranolol [3 (1 naphthoxy)-1 isopropylamino-² propanol] or 12.5 mg INPEA [1-(4 nitrophenyl) 2 isopropylaminoethanol] was injected intravenously Another interruption of stimulation followed ten

Table II *The influence of isoprenaline i v on atrial rate before and after propranolol and INPEA i v*

Patient	Isoprenaline (μg)	Atrial rate: Before isoprenaline	Atrial rate: After isoprenaline	Drug administered	Atrial rate: Before isoprenaline	Atrial rate: After isoprenaline
K Ö ♀	12	90	114	Propranolol	90	85
A E. ♀	8	90	100	Propranolol	77	83
T F ♀	6	80	90	Propranolol	65	70
C C. ♂	6	80	87	Propranolol	68	80
K Ö ♀	12	92	110	INPEA	88	94
R J ♂	8	84	111	INPEA	89	100
C. L. ♀	8	100	111	INPEA	105	112
G S ♀	8	70	78	INPEA	65	72
O E. ♂	12[a]	50	65			
R R ♂	12[a]	89	1.0			
H W ♂	12[a]	82	97			
H H ♀	12[a]	78	93			
G W ♂	12[a]	100	120			

[a] Produced no idioventricular beats.

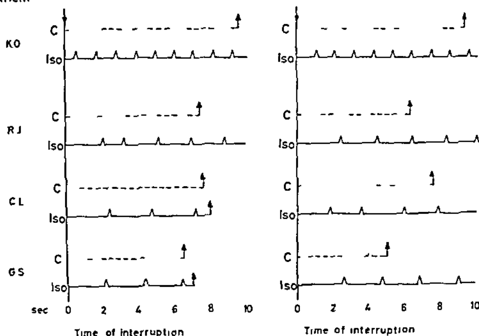


Fig 1 Isoprenaline-induced idioventricular rhythm after interruption of electrode pacemaker stimulation Repeated study in each patient 5 min after first isoprenaline injection. *C* control *Iso* isoprenaline injected i v 45 sec before interruption ∧ ventricular beat

min later After another five min the patient was again given isoprenaline intravenously in the initial dose and the interruption procedure was repeated If there was still an effect with isoprenaline further doses of propranolol (1 mg) or INPEA (12.5–25 mg) were given, later followed by isoprenaline and cessation of stimulation

The ECGs (four leads) were continuously recorded by a four-channel ink writing Elema Schonander Mingograph 4 B The atrial and ventricular frequencies were determined from the ECGs

MATERIAL

A total of 13 experiments were carried out in 1 patients aged between 56 and 8? They had been treated with pacemaker for half a year to seven years (Table I) Examinations with the methods described above were done normally when a routine exchange of battery was made No patient suffered from asthma renal insufficiency or anemia. Except for one woman with a B P of 190/110 mm Hg at rest all the patients were considered normotensive (Table I) Their relative heart volumes varied between 340 and 750 ml/square meter of body surface area (Table I) Their drug therapy (Table I) was not altered on the day of the study Propranolol was administered to four patients, and INPEA also to four

RESULTS

The dose of isoprenaline needed to produce idioventricular rhythm was 6 μg in two 8 μg in four and 12 μg in one patient In five patients 12 μg of isoprenaline did not produce any idioventricular activity However the atrial frequency increased in those by an average of 19 beats/min (Table II)

In four experiments where two separate injections of the same dose of isoprenaline were given one after the other identical effects on the ventricular activity were produced on both occasions during the interruption of the pacemaker stimulation (Fig 1)

After *propranolol* isoprenaline provoked isolated ventricular complexes in two patients while no response occurred in the other two patients (Fig 2) In one patient (A E Fig 2) spontaneous ventricular activity appeared during the interruption before isoprenaline was given Propranolol also blocked this response The experiments with

PATIENT PROPRANOLOL

KÖ C Iso 1 mg

AE C Iso 2 mg

TF C Iso 2 mg

CC C Iso Iso* 1 mg

sec 0 2 4 6 8 10

Time of interruption

0 2 4 6 8 10

Time of interruption

Fig 2 The influence of propranolol on isoprenaline induced idioventricular rhythm after interruption of electrode pacemaker stimulation (*C Iso* ∧ see text for Fig 1 *Iso** isoprenaline injected 1 0 sec before interruption)

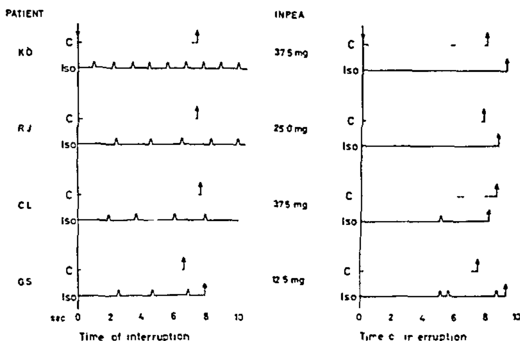


Fig 3 The influence of INPEA on isoprenaline-induced idioventricular rhythm after interruption of electrode pacemaker stimulation. (*C Iso* ∧ see text for Fig. 1)

INPEA gave results quite similar to those with propranolol (Fig 3)

The maximum increase in the atrial frequency after isoprenaline averaged 13 and 16 beats/min respectively in the two series before propranolol and INPEA were administered (Table II) The increases were reduced after propranolol to 5 beats/min and after INPEA to 8 beats/min (Table II) Propranolol in contrast to INPEA administration in itself resulted in a decrease in atrial frequency (Table II)

DISCUSSION

Animals have hitherto had to be used to study the effect of drugs on the conductive system and the automaticity of the ventricular muscles (3 4 10 11) Patients with intracardiac electrical stimulation from an external pacemaker the frequency and voltage of which can be varied offer possibilities of such studies in man The safe procedure used in the present investigation may be the only way of detecting and exposing otherwise unrecognized but nevertheless significant effects of drugs on cardiac automaticity in man A similar method for such studies of the effect of drugs on cardiac automaticity has recently been described (1)

The present observation that the ventricular automaticity increases after isoprenaline is in accordance with findings in animals (3 13) The responses rapidly disappeared but could then be elicited again One finding was unexpected five out of twelve patients did not respond with idioventricular activity to doses of isoprenaline that produced significant increases in the sino atrial rate It is difficult to explain the decreased responsiveness of the ventricles in these patients It has earlier been reported that the dose of isoprenaline necessary to arouse a ventricular pacemaker varies widely from patient to patient (13)

The observation that propranolol blocked the ventricular rhythm induced by isoprenaline might indicate that the effect of the sympathomimetic amine is mediated by adrenergic beta receptors Though propranolol is a potent beta receptor blocking agent it also possesses significant local anesthetic properties (8 12) Ventricular automaticity is inhibited by local anesthetic drugs (7) The blockade produced by propranolol might therefore at least in part be explained by its anesthetic property Additional experiments were thus required to study the significance of the involvement of blockade of beta receptors in the response

An adrenergic beta receptor blocking substance without significant local anesthetic properties would serve that purpose The compound INPEA seems to meet these demands (8 12) INPEA like propranolol was found to block the idioventricular rhythm produced by isoprenaline It thus appears that beta adrenergic receptors are significantly involved in the control of ventricular automaticity in man as in animals

Propranolol and INPEA both block the effect of isoprenaline on the ventricular automaticity in doses that are smaller than those which inhibit the isoprenaline induced increase in the sino atrial rate This would mean that treatment with even small doses of beta receptor blocking substances may depress an increase of ventricular automaticity produced by augmented sympatho adrenal activity Clinically this means that beta receptor blocking drugs may impede emergency resuscitation with isoprenaline and other beta mimetic agents in patients with ventricular standstill

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ON THE OCCURRENCE OF THE α ISOMER OF DIPHOSPHOPYRIDINE NUCLEOTIDE AND ON THE DEHYDROGENASES ACTIVATED BY IT IN LEUCOCYTES AND NEOPLASTIC TISSUES

A Metabolic Error

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Abstract The α isomer of pyridine nucleotides has hitherto been regarded as an inactive form not encountered in tissues

The α and β isomers of pyridine nucleotides were determined in connection with their synthesis in some neoplastic tissues and in the white blood cells of normal persons and selected patients as the CN complex, and by paper chromatography The α isomer could be detected by the former method in about half the samples studied With chromatography α diphosphopyridine nucleotide was likewise demonstrated in about half the tests and the presence of α triphosphopyridine nucleotide was established in approximately a fifth The activating influence of the α and β isomers of diphosphopyridine nucleotide on a total of 7 dehydrogenases was also studied In all the groups examined the α isomer activated lactic and malic dehydrogenases and cytochrome c reductase often very strongly The effect of the α isomer occasionally exceeded that of the β isomer The α isomer was generally capable of inhibiting the dehydrogenase activating ability of the β isomer sometimes very markedly Although the phenomenon established is common it is probably a metabolic error

The present work is a study of the occurrence of the α isomer of pyridine nucleotides (DPN TPN) in human leucocytes in normal persons in persons affected with certain diseases and in some neoplastic tissues of man and rat In addition the activating effect of α DPN and of the corresponding β isomer were determined at the same time According to the literature the α isomer is not encountered in human or animal tissues and is completely inactive in them

MATERIAL AND METHODS

Sprague Dawley rats were used in the study Some of them had been inoculated when young with chloroma (experimentally transmitted form of rat leukaemic tumour) tissue originally supplied by Dr Harry Shay of Philadelphia The rats were sacrificed 41–145 days after inoculation Hepatomas were induced by feeding the rats with 4-dimethylaminoazobenzene and using a specified diet (14)

Study of hepatoma and of cancer and chloroma tissue

The rat was anaesthetized with ether the renal vein was severed and cold physiological NaCl solution was injected into it The tissue to be examined was then frozen rapidly and the procedure employed for the leucocytes and nuclei was applied

Isolation of leucocytes and nuclei

Leucocytes were separated from 80 ml of blood by the method of Minot and Burnett (15)

The nuclei were isolated according to Brandster and Morton (6)

The sediments were suspended in a volume of physiological NaCl solution (containing titriplex II) five times the weight of the leucocytes or the nuclei Saccharose containing solution appeared to disturb DPN synthesis

The nuclear suspension was examined for DPN pyrophosphorylase Other enzymes were studied in the supernatant

DPN pyrophosphorylase test (6)

Reaction mixture

0 95 ml 0 25 *M* glycylglycine buffer pH 7 4
2.5 mg NMN
0 25 ml 0 06 *M* ATP pH 7 4
0 30 ml 0 13 *M* MgCl
0 50 ml 0 5 *M* NA
0 10 ml 0 4 *M* NaF
0 5 ml of the suspension to be studied
2 6 ml

After incubation for 0 min at 38 C, the reaction was stopped by adding 0 4 ml of 15% perchloric acid Centrifugation was then carried out, and the supernatant recovered

Table I *Pyridine nucleotide synthesis in leucocytes and some tissue nuclei by DPN pyrophosphorylase measured as CN complex and by chromatography*

327 mμ – β DPN + β TPN 333 mμ – α DPN + αTPN 340 mμ – α + β DPNH and α + β TPNH *Results transformed* to μg DPN mgN/30 min N = nitrogen ( ) = weak positive

Cases	327 mμ	333 mμ	340 mμ	Chromatography
"Normal"	0	0	0	Not done
persons	0	0	0	Not done
age				
2? 31 years	0	0	0	β DPN
	0	0	0	0
	0	0	0	α TPN (β DPN) (β TPN)
	0	0	0	α TPN (β DPN)
	0	0	0	α DPN (β DPN)
	0	12	0	α DPN β DPN (β TPN) β DPNH
	0	0	0	α DPN β DPN β DPNH
Neuro-	0	0	0	β DPNH (β TPN)
circulatory	290	780	735	α TPN (β DPN)
asthenia	0	0	0	β DPN
	83	0	0	β TPN
Psoriasis	210	220	430	α DPN β DPNH
	390	705	540	α DPN (β DPN)
	0	0	0	α DPN
Myeloid	0	925	1315	Not done
leukaemia	224	540	490	Not done
	1300	2930	2440	Not done
	127	220	307	α DPN β DPN
	0	0	0	0
	Not done	Not done	Not done	Not done
	Not done	Not done	Not done	Not done
	Not done	Not done	Not done	Not done
	50	39	0	β TPN
	8 0	370	760	α DPN
	112	350	708	α DPN β DPN
Lymphatic leukaemia	38	130	140	0
Polycythaemia	0	0	0	0
vera	0	0	0	Not done
	0	0	1300	β DPNH α DPN
Myeloma	310	690	650	α DPN β DPN
Lymphogran	580	845	740	α DPN
maligna	0	300	785	β DPN α DPN β TPN
Chloroma	0	0	0	α DPN α DPNH
tissue	Not done	Not done	Not done	Not done
	22	36	55	Not done
	0	0	0	β DPNH
	0	0	0	β DPN
	Not done	Not done	Not done	Not done
	0	0	0	α DPN β DPN
	0	0	0	α DPN
Cancer tissue				
C. uteri	0	0	0	β DPN
C. vesic. urin.	0	0	0	Not done
C. ventriculi	360	840	650	(β DPN)
C. renis	0	0	0	α DPN
C. ventriculi	0	0	0	β DPN
C. uteri	94	148	0	β DPN α DPN
HELA cells	41	0	0	β DPN
	193	390	260	β DPN
Hepatoma	170	586	495	α + β DPNH
tissue	49	68	20	α + β DPNH
	23	0	0	β TPNH
	140	384	415	α TPN
	0	57	44	α TPN
	54	136	145	α TPN
	0	0	0	α TPN
	0	0	0	β TPN
	40	63	74	α TPN β TPN
	0	0	0	α TPN β TPN
	46	35	0	α DPN β DPN
	0	99	97	α DPN
Normal rat	32	90	120	β DPN α DPN β DPNH
liver	0	134	0	α DPN β DPN

CN complex measurements (1 10)

Into the test tube was introduced

0.5 ml of the above supernatant to be studied
1.5 ml KCN (6.5 g/100 ml)
6.0 ml H_2O

The mixture was allowed to stand for 10 min Measurements were made at wavelengths of 327 mμ 333 mμ and 340 mμ For β DPN-CN the absorption maximum occurs at a wave length 327 mμ for α DPN-CN at 333 mμ and for DPNH-CN at 340 mμ The same values are obtained with TPN The CN complex of the DPN $(T-T')-(R-R')$ formed was calculated in μg per mg of nitrogen (T = incubated and T' = unincubated supernatant R = incubated and R' = unincubated reagent control)

Chromatography for the determination of α DPN and α TPN (7 11)

One ml of the incubated supernatant was introduced into three test tubes (unincubated supernatant into one tube) two of which were neutralized with 4 N KOH with neutral red as indicator and centrifuged if necessary From one tube (I) β DPN was removed with alcohol dehydrogenase and from the other (II) β TPN with isocitrate dehydrogenase

The following substances were added Into tube I (7) 2 ml of buffer pH 8.7 ($Na_4P_2O_7$ 10 H_2O 3.3 g semicarbazide HCl 0.83 g glycine 0.17 g, 1 ml of absolute ethanol 3.3 ml of 2 *N* NaOH made to 100 ml with water) and 0.0_ ml ADH Into tube II (3) 1.75 ml of buffer of 0.05 *M* triethanolamine pH 7.45 0.1 ml 0.1 $MgCl_2$, 0.1 ml 0.04 *M* DL isocitrate 0.05 ml ICDH

After incubation for 30 min at 25 C the reaction stopped by adding 15 per cent perchloric acid until liquid turned permanently red After centrifugation, four tubes were treated with ion exchanger Permu "indicator biodem nrolit" The Permutit Co Ltd L don. The ion exchanger had been washed in ... water and dried before use This ion exchanger both acids and bases (salt) Filtering and cen r

were carried out and the solution was dried overnight over PO at +4 C in a vacuum desiccator (the vacuum was formed with a vacuum pump until the liquid froze in the tubes)

The residue was dissolved in 0 1 ml of distilled water and pipetted onto chromatography paper Schleicher & Sohn no 2043 b the paper having previously been washed with distilled water for 24 hours by the descending system and dried

The ascending system (7) was applied in the separation, with pyridine water (2 1) as solvent for about 18 hours After drying the paper was exposed to ultraviolet light and the fluorescing spots (DPNH and TPNH) recorded the paper was then sprayed with KCN solution (6.5 g/100 ml) and dried It was re-exposed to ultraviolet light and the additional fluorescing spots (DPN and TPN) were recorded

By adding β DPN β DPNH α DPN or α DPNH the following enzymes were determined from the supernatant (mitochondria and microsomes) obtained at isolation of the nuclei malic dehydrogenase (17) lactic dehydrogenase (4) cytochrome *c* reductase (16) alcohol dehydrogenase (?) glycerol dehydrogenase (2) glycerophosphate dehydrogenase ( ) glutamate dehydrogenase (5) and butyrate dehydrogenase (8)

Nitrogen determination was performed on 0.5 ml of the nuclear suspension and separately on the supernatant, using Kjeldahl s method

The following substances were used in the tests β DPN and β DPNH from C F Boehringer & Sohne Mannheim, α DPN α DPNH and nicotine amide mononucleotide (NMN) from Sigma Chemical Co St. Louis, Missouri, USA nicotine amide (NA) from Hoffmann-La Roche Basel Switzerland alcohol dehydrogenase malic dehydrogenase lactic dehydrogenase and isocitrate dehydrogenase from C F Boehringer & Sohne Mannheim

The amount of test coenzyme added in the experiments was 0 05–0 1.5 ml of the stock solution which contained 100 mg of test coenzyme per 10 ml of water and 3 ml of the incubation mixture The reaction was followed until it stopped The result of the blank test, in which no test coenzyme was added was subtracted from the reading The determinations were made in duplicate The activity of the enzymes was found to be linear for five to ten minutes

CONTROLS

The absorption of pyridine nucleotides was measured with a Beckman spectrophotometer The full absorption curves of the oxidized and reduced forms of β TPN and of the α and β isomers of DPN as well as of their CN complexes, have been plotted In this way it was established that each absorption maximum (Table I) corresponds to the values reported in the literature (1)

The presence of the different forms of the abovementioned pyridine nucleotides was established using the chromatographic method described from the positions of the spots of each of the substances on the chromatograph paper This result is also consistent with the reports in the literature

Table II *Synthesized α and β isomers of pyridine nucleotides described in Table I*

Cases	No	CN complex β DPN β TPN	CN complex α DPN α TPN	Chromatography α DPN	Chromatography α TPN
Normal persons	9	0	1	3	2
Neurocircul asth	4	2	1	0	1
Psoriasis	3	2	2	3	0
Leukaemia	9	7	8	3	0
Polycythaemia	3	0	0	1	0
Myeloma	1	1	1	1	0
Lymphogranul	2	1	1	3	0
Chloroma	6	1	1	3	0
Human cancer	6	2	2	2	0
HELA-cells	2	2	1	0	0
Hepatoma	12	7	8	6	7
Normal rat liver	2	1	2	2	0
Total	59	26	28	27	10

It must be remembered that the pyridine nucleotide preparations obtainable are not pure but always contain both isomers but in general the α isomers contain more β isomer than the β isomers contain α isomer

No activity was established with the three pure Boehringer enzymes (alcohol dehydrogenase lactic dehydrogenase and malic dehydrogenase) when α DPNH was added but it was clearly seen when the corresponding β isomer was employed

RESULTS

The results are presented in detail in Table I A total of 28 tissue samples and the leucocytes of nine normal subjects and 22 patients were examined The α isomer of pyridine nucleotides was detected as the CN complex in 28 of the 59 cases (Table II) rarely in normal persons mostly in cases of leukaemia and hepatoma Chromatography on the other hand revealed α DPN in 27 and α TPN in ten tests The amount of the CN complexes present was mostly proportional to the intensity of the pyridine nucleotide spots demonstrated chromatographically It should be mentioned that the patients with leukaemia and polycythaemia had received various treatments for their diseases

In 49 cases it was possible to activate LDH with α DNPH in 44 incubation tests (Tables III and IV) CR in 30 and MDH in 32. The highest activity value when α DPNH was added was 2820 g for LDH 6000 for CR and 3660 for MDH The activation produced by the α isomer was

Table III *Total coenzyme consumption in μg/mg N/20 min in mitochondrial + mitochondrial supernatant enzymes by addition of pyridine nucleotides α and β isomers*
LDH = lactic dehydrogenase CR = cytochrome c reductase
MDH = malic dehydrogenase
N = nitrogen

Cases (leucocytes)	Coenzyme added	Enzymes LDH	CR	MDH
*Normal persons, age 22-31 years	Not done	Not done	Not done	Not done
	Not done	Not done	Not done	Not done
	β DPNH	730	30	
	α DPNH	230	0	Not done
	β DPNH	500	0	
	α DPNH	0	0	Not done
	β DPNH	1000	56	1970
	α DPNH	89	220	164
	$\alpha+\beta$ DPNH	328	110	1640
	β DPNH	325	0	20[illegible]0
	α DPNH	138	0	34
	$\alpha+\beta$ DPNH	86	0	1125
	None	0	0	0
	β DPNH	4300	178	7000
	α DPNH	178	224	224
	$\alpha+\beta$ DPNH	1000	90	1875
	None	250	0	128
	β DPNH	7400	190	17 000
	α DPNH	700	510	575
	$\alpha+\beta$ DPNH	24[illegible]0	385	4[illegible]00
	None	0	0	100
	β DPNH	348	0	1690
	α DPNH	595	75	150
	$\alpha+\beta$ DPNH	150	25	50
Neurocircul. asthenia	β DPNH	1460	183	Not done
	α DPNH	2100	275	Not done
	β DPNH	4700	134	1700
	α DPNH	168	33	0
	$\alpha+\beta$ DPNH	1540	0	33
	β DPNH	7450	825	13 700
	α DPNH	360	600	7[illegible]0
	None	0	78	0
	β DPNH	260	52	2270
	α DPNH	365	130	935
	$\alpha+\beta$ DPNH	0	6	1880
Psoriasis	None	0	13	0
	β DPNH	925	66	1650
	α DPNH	79	86	105
	$\alpha+\beta$ DPNH	105	13	825
	None	0	0	144
	β DPNH	260	107	9400
	α DPNH	640	214	750
	$\alpha+\beta$ DPNH	785	35	8550
	β DPNH	110	150	0
	α DPNH	1800	165	3660
	$\alpha+\beta$ DPNH	1[illegible]4	155	124
Myeloid leukaemia	β DPNH	1004	Not done	0
	α DPNH	Not done	Not done	0
	β DPNH	445	Not done	985
	α DPNH	Not done	Not done	Not done
	β DPNH	550	Not done	750
	α DPNH	Not done	Not done	Not done
	β DPNH / α DPNH	Not done	Not done	Not done
	β DPNH	1000	Not done	Not done
	α DPNH	184		
	β DPNH	380	Not done	Not done
	α DPNH	1330		
	β DPNH	750	Not done	Not done
	α DPNH	785		
	β DPNH	490	Not done	Not done
	α DPNH	845		
	β DPNH	1400	Not done	Not done
	α DPNH	450		
	None	0	0	0
	β DPNH	1120	82	6800
	α DPNH	650	745	490
	$\alpha+\beta$ DPNH	163	0	2[illegible]00
	None	0	37	0
	β DPNH	850	63	7750
	α DPNH	160	127	63
	$\alpha+\beta$ DPNH	127	37	770
	β DPNH	1000	Not done	Not done
	α DPNH	1080		
Polycythaemia vera	β DPNH	2360	113	5900
	α DPNH	2870	6100	2600
	β DPNH	3335	140	4550
	α DPNH	430	180	370
	$\alpha+\beta$ DPNH	960	0	1800
	None	0	0	0
	β DPNH	125	0	2300
	α DPNH	104	0	1150
	None	52	78	Not done
Myeloma	β DPNH	208	0	1640
	α DPNH	130	0	734
	$\alpha+\beta$ DPNH	104	0	5[illegible]0
Lymphogranul maligna	None	196	33	0
	β DPNH	130	33	7[illegible]0
	α DPNH	130	98	590
	$\alpha+\beta$ DPNH	65	33	650
	None	318	158	63
	β DPNH	190	0	900
	α DPNH	318	32	1780
	$\alpha+\beta$ DPNH	254	0	890
Chloroma tissue	β DPNH	2700	36	Not done
	α DPNH	675	55	
	β DPNH	2000	21	950
	α DPNH	157	35	285
	β DPNH	1960	27	1300
	α DPNH	1000	36	7[illegible]
	β DPNH	2910	18	4150
	α DPNH	374	28	93
	β DPNH	2465	16	1360
	α DPNH	65	Not done	Not done
	β DPNH	2800	0	9700
	α DPNH	330	67	705
	None	19	14	19
	β DPNH	2750	0	7000
	α DPNH	19	10	10
	$\alpha+\beta$ DPNH	128	0	130
	None	9	0	9
	β DPNH	2130	5	11 500
	α DPNH	80	18	18
	$\alpha+\beta$ DPNH	430	5	18
Human cancer tissue				
C. uteri	β DPNH	1210	Not done	1140
C. vesic. urin.	β DPNH	1345	Not done	Not done
C. ventriculi	β DPNH	1700	0	5700
	α DPNH	670	27	670
C. renis	β DPNH	1650	0	1470
	α DPNH	38	0	6

Cases (leucocytes)	Coenzyme added	Enzymes LDH	CR	MDH
C. ventriculi	β DPNH	1680	0	4250
	α DPNH	43	0	43
	None	49	6	0
C. uteri	β DPNH	135	0	8.5
	α DPNH	62	6	25
	α+β DPNH	0	0	130
HELA-cells	β DPNH	1060	62	1000
	α DPNH	79	88	62
	α+β DPNH	855	17	1040
	β DPNH	3600	27	3900
	α DPNH	320	130	370
	α+β DPNH	2140	0	2000
Hepatoma tissue	β DPNH	512	Not done	1380
	β DPNH	45	Not done	136
	β DPNH	950	Not done	9 0
	β DPNH	2230	Not done	Not done
	β DPNH	700	Not done	Not done
	α DPNH	120		
	β DPNH	120	Not done	Not done
	α DPNH	57		
	β DPNH	670	Not done	Not done
	α DPNH	0		
	β DPNH	0	0	0
	α DPNH	0	Not done	Not done
	β DPNH	950	0	
	α DPNH	15	Not done	Not done
	β DPNH	1115	16	1100
	α DPNH	0	Not done	Not done
	None	94	38	0
	β DPNH	5600	0	5550
	α DPNH	1300	47	140
	α+β DPNH	2650	0	520
	None	63	84	84
	β DPNH	2360	0	6680
	α DPNH	7	63	168
	α+β DPNH	460	0	460
Normal rat liver	None	82	49	74
	β DPNH	1740	8	2900
	α DPNH	115	16	95
	α+β DPNH	345	0	270
	None	32	0	0
	β DPNH	4200	0	3075
	α DPNH	128	64	255
	α+β DPNH	1000	0	385

Table V *α DPN activation of some dehydrogenases outside of the energy metabolism*

Cases in Table II

Dehydrogenases	No	α DPN activation Positive	Negative
Alcohol	59	20	39
Glycerol	59	21	38
Glycerophosphate	57	11	46
Glutamate	0	1	19
Butyrate	40	5	35
Total	235	58	177

stronger than that provoked by the β isomer in 37 tests performed with the three enzymes Some times dehydrogenase was activated only by the α isomer When both isomers were added to the same incubation solution activity was less strong than that elicited by the β isomer *alone* in 48 of the experiments The decrease varied greatly ranging from large to slight or almost absent The largest decrease in β DPNH activity was from 15 000 μg to 18 in a case of polycythaemia

The β isomers sometimes produced activation of a dehydrogenase which appeared to exceed even the normal value of the enzyme In the material as a whole the greatest rise in activity provoked by the two isomers of pyridine nucleo tides was mostly achieved in tests with LDH

The activating effect of α DPN was also tested with five enzymes (alcohol glycerol glycero-phosphate glutamate and butyrate dehydrogenase) not participating in energy metabolism proper

Table IV *Activated dehydrogenases after addition of α DPNH and their relation to β DPNH-activation*

Cases	No	α DPNH activated LDH	CR	MDH	α activation > β activation	α DPNH Suppress β DPNH
Normal persons	7	6	4	5	4	11
Neurocircul asth	4	4	4	2	4	6
Psoriasis	3	3	3	3	6	5
Leukaemia	8	7	2	2	6	3
Myeloma	1	1	0	1	0	2
Lymphogranul.	2	2	2	2	3	1
Chloroma	8	8	7	6	7	4
Human cancer	4	4	2	4	2	2
HELA-cells	2	2	2	2	2	5
Hepatoma	8	5	2	3	1	4
Normal rat liver	2	2	2	2	2	5
Total	49	44	30	32	37	48

(Table V) In about a quarter of the incubations the result was positive it was generally fairly low less than 100 μg/mgN/20 min However one value of 1470 was recorded for alcohol dehydrogenase in a case of leukaemia and 990 was recorded in polycythaemia

In blank tests without addition of the coenzyme no activity was demonstrated in about half the incubations The values were generally below 100 in the remaining tests but exceeded this value in some experiments It proved impossible to activate α ketoglutarate dehydrogenase with either the α or β isomer of pyridine nucleotides in 19 experiments performed in different cases According to the literature the enzyme should be isolated first

DISCUSSION

Is the hitherto unknown metabolic phenomenon described in the foregoing normal or pathological? Its occurrence in normal" subjects as well as in the rest of the material might perhaps be taken as support for the first alternative Yet the many observations presented here tend to suggest that the appearance of the α isomer of pyridine nucleotides and the activation of certain dehydrogenases by it are by nature alien to the normal organism

There were indications of accelerated cellular activity in connection with the appearance and activating effect of α isomer on dehydrogenases Frequently the cells had no pyridine nucleotide depots as is the case in normal tissue Enzyme activity was not elicited until the coenzyme was added The phenomenon might perhaps be due to accelerated consumption of the coenzyme

Human leukaemia leucocytes as well as chloroma tissue seem to have retained the capacity for oxidative phosphorylation which disappears as the leucocytes mature The phosphorylation may be of glycolytic origin in experiments with weak or absent oxygen consumption (9 13) The α isomer may have a stimulating influence on metabolic processes of bone marrow cells that are normally stabilized

The metabolic error described might perhaps also provoke some other diseases in which stimulation of cellular activity is involved One way of clarifying this point would be to synthesize an antimetabolite for α isomer and test its effect a therapeutic remedy

An earlier publication (12) mentions that oxidative and glycolytic phosphorylation are activated or accelerated in many neoplastic tissues when certain substrates are used by the addi not only of β DPN but also of β TPN or , quently the latter alone This too suggests change of the dehydrogenases in the cases studied

It would have been desirable to study the of α TPN on dehydrogenases known to be a tivated by TPN However α TPN has not , obtainable from any factory or laboratory

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DIRECT CURRENT COUNTERSHOCK COMPLICATIONS

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Abstract Conversion with direct current countershock was attempted in 215 patients with arrhythmias the majority of whom (81 9%) had atrial fibrillation There was a fairly high frequency of complicating ventricular ar rhythmias (25%) probably due to medication with digi talis and/or quinidine Thromboembolism was only noted in one case and pulmonary edema in three cases

In 1962 Lown et al introduced the direct current (DC) countershock technique to terminate un desirable arrhythmias (13) Since then many re ports have been published dealing with the ef fectiveness and advantages of this technique (3 7 8 9 10 14 16 18 20 24 33) As in most new therapeutic methods the indications will be come clearer as the results and the complications become better known The purpose of this study is to report the complications in our DC treated material

MATERIAL

Two hundred and fifteen patients were treated with DC countershock from Augu t 1963 to April 1967 Altogether 3?3 attempts at elective convers ons were performed and a total of 775 shocks were administered The material consists of 111 females and 104 males The mean age was 51 3 years (range 18 to 81 years) The distribution of the underlying heart diseases and of the different arrh thmias is shown in Table I

METHOD

Almost every patient has been treated with dicumarol prior to the conversion The shock has not been ad m nistered until the pat ent has had a serum le el of 10–5% Trombotest duri g a ten-day period at least. The aim has also been to avoid h popotassemia during the shock

Up to January 31 1966 d gitalis was as a rule main tained dur ng the countershock Since that time we have discont nued d goxin 48 hours and digitoxin 7? hours prior to the shock Quin d ne bisulphate with sustained release (Duretter® Hassle Gothenburg Sweden) in maintenance dose schedule aiming at a serum level of 5–7 mg/l has been given in most patients prior to and during the convers on Since September 30 1966 we have not given quinidine prior to the shock We have not given the DC shock on an ambulatory basis

A synthetic morphine preparation (Petidin®) and promethazine (Lergigan®) in combination were used as premedication

General anesthesia with an intravenous rapid acting barbiturate (Pentothal®) sometimes combined with suc cinylcholine (Celocurin®) has been used Pure oxygen has been given by manually ass sted ventilation and if more shocks were necessary nitrous oxide and oxygen have been given

We have used the Cardiac Synchronizer (Corbin Farns-worth Company) in most cases. Some conversions have been performed with other cardioverters (Elema Scho-nander American Optical Company and Electrodyne)

The limb lead with the tallest R or S wave has been used and the shock has been released as soon as possible after the peak of the wave (15)

We have placed the electrodes in the antero-lateral position (one at the 2nd intercostal space to the right of the sternum and the other in the mid axillary line at the 4th intercostal space) In the earliest cases we used the electrode placement originally used by Lown (one electrode at th base of the heart and the other at the apex area) and in some others an antero-posterior place ment with the posterior electrode at the interscapular area

The available energy levels have been 80 100 ?00 300 and 400 Watt seconds (Ws) As a rule we have not given more than four shocks at the same attempt During the procedure the patient has also been connected to a 4-channel direct writing electrocardiograph

RESULTS AND COMMENTS

The different complications discussed are seen in Table II The technical problem involved has been to avoid alternating current disturbance which is important in hospitals nowadays with their increasing amount of electrical equipment. Rarely has it been difficult to avoid an AC

Table III *Cases with serious arrhythmias*

Abbreviations as in Table I

Case	Age (y)	Sex	Diagnosis	Operation date	Heart size (ml m² BSA)	Rhythm	Duration	Digitalis up to cardio-version	Quinidine level (mg/l)	Potassium (mEq/l)	DC shock date	Outcome of DC shock
1	54	♀	MS (*op*)	4/1964	740	AF	1 year	+	8 8	4 1	5/1964	VES – VF – VES – SR
2	53		MS MI		1110	AF	5 years	+	—	4 2	11/1964	SR + VES – *VT* – SR
3	51	♂	MS (op) AI	11 1963	7 0	AF	5 years	+	4 8	4 7	12/1964	VT *VF* – SR + VES
					7 0	AF	2 weeks	+	2 0	5 0	?/1966	SR
4	42		MS (*op*) AI	3/1963	680	AF	6 weeks	+	7 6	4 0	10/1965	VT – *VF* – SR
5	57		MS (op)	8/1964	740	AF	1 years	–	2 6	4 0	10/1965	SR + VES – VT – SR
6	62	♂	ASHD	—	490	AFl	?	–	—	—	1?/1965	NR – SR – VF – *VT* – SR
7	61	♂	ASHD	—	750	AF	½ year	–	3 2	4 0	3/1966	No change
					750	AF	½ year		4 6	4 0	3/1966	SR + VES – VT – *VF* – AF
8	61		MS MI	—	570	AF	3 years		6 4	2 7	6/1966	VES – *VT × 10* – SR

Italics indicate the need of another direct current countershock Serum quinidine was determined by the method of Balatre in a modification by Hamfelt and Malers (Acta Soc. Med upsalien 68 181 191 1963)

incidence of embolism was 1 1% Reinikainen et al reported two patients with embolism out of 63 with 116 conversions (1 7%) (24) Korsgren et al did not observe any peripheral embolism "during or immediately after the delivery of countershock" (10) but the length of the time of observation is not given

In the present study we had one patient with cerebral embolism occuring two days post conversion This patient was a 61 year-old woman with a combined aortic and mitral valvular lesion, having atrial fibrillation She developed a hemiplegia with persisting severe neurological sequelae In spite of careful control of the serum level of *trombotest she was shocked by mistake at a* level of 39% We also had another case with peripheral embolism 18 days after the conversion The latter patient had also a previous history of embolism and the connection with the countershock in her case would be difficult to substantiate The percentage in our material (only the first patient counted) is then very low namely 0 3

In general our impression is that the incidence of embolism is lower in series in which prophylactic anticoagulation therapy has been administered This impression seems to be adequate when comparing some series from the USA In spite of many differences the present opinion in the USA is to give dicumarol only when an embolic episode has occurred within the last two months (4)

The investigation by Szekely might be propriate to review here He found that dicumarol significantly reduced the incidence of em in patients with rheumatic valvular disease atrial fibrillation (30) This clinical study rather extensive and the results impressive

Arrhythmias

We have changed electrode placement in an tempt to reduce undesirable arrhythmias after the shock The aim has been to decrease electrical field over the ventricles It is however difficult to prove that one placement superior to the other It may be of interest mention that in a few cases we have been able to terminate an arrhythmia with the posterior position of the electrodes where positions have failed This position has claimed to be more effective (18)

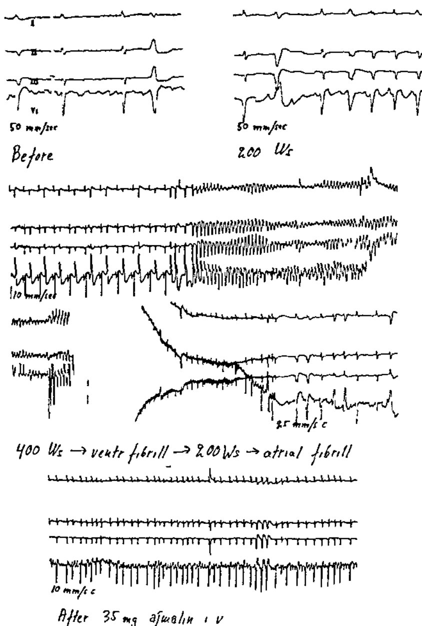


Fig 2 ECG prior to and immediately after the countershock (case 7 in Table III)

In the immediate post-conversion period there is a great incidence of arrhythmias as well as A V blocks We found nodal rhythm mostly transitory in 7 5 % Second degree A V block and wandering pacemaker were registered in about 3 % Repeated ventricular and supraventricular premature beats occurred in more than 20 % In one case we have had asystole (about 10 seconds) but the cause was probably aspiration Resuscitation was successful

As serious complication we have considered ventricular tachycardia and ventricular fibrillation which occurred in 2 5 without mortality The corresponding percentage in the earlier mentioned report by Morris et al was 1 1 with a mortality of 0 6 (18) Likoff and coworkers as well as other authors have divided the post conversion ventricular arrhythmias into two groups one with ventricular tachycardia or fibrillation occurring immediately after the shock in which there are probably some cases of shock released in the vulnerable phase This group is

relatively benign In the other group the arrhythmia started half a minute later or more and led to death in uncontrollable fibrillation (22) The cause of this probably in most cases is digitalis and/or quinidine

Our cases with post shock ventricular tachycardia and fibrillation are seen in Table III

The high percentage of ventricular arrhythmias is in our series strikingly connected with quinidine and/or digitalis One report stresses the possibility of an increase of the toxicity of quinidine by the countershock (2) We have two cases (nos 3 and 7 in Table III) who are their own controls where the relationship of the serum level of quinidine to the complicating ventricular arrhythmia is suggestive

Only one case (no 4) developed ventricular tachycardia immediately after the shock In the others it occurred later Since we have discontinued quinidine and digitalis prior to the shock we have not had any ventricular tachycardia or fibrillation In one case (no 6) with ventricular tachycardia the patient had not received quinidine or digitalis prior to the shock Our findings do not confirm the prognostic significance of the time interval (22)

ST Changes

nally we have not seen the ST changes as often some other authors (17 20) The frequency in r material does not exceed 3% One of the planations of the different incidence in earlier ports may be the selection of leads on the ectrocardiograph during the conversion We e the limb leads and V_1 which are suitable for the study of arrhythmias but perhaps not as good for evaluating the ST changes

Another reason may be that initially they were not as carefully looked for

The ST changes in our material have all been very transient and none existed on the following day As a rule these patients had had more than one shock but there are exceptions We have not seen any T wave inversion following the ST changes This seems to occur very seldom and it is an open question whether these changes reflect a myocardial injury (24)

Transaminases

We have seen post-conversion elevation of the transaminases (serum glutamic oxaloacetic transaminase SGOT and serum glutamic pyruvic transaminase SGPT) in 7% There is no relationship between the elevation of the transaminases and the presence of ST changes or the energy given in our material As to the transaminase level prior to and after the shock some reports have a higher incidence of elevation (1 24 28) When studying other enzymes than SGOT and SGPT such as aldolase and lactic dehydrogenase the increase in the post shock levels of serum enzymes is evident Increased values of aldolase seem to be more frequent than those of transaminases and lactic dehydrogenase (28)

CONCLUSION

The direct current countershock has proven its great value However it is important to know the different complications In our review we have discussed some of them In one respect our material is different from others as we have more ventricular arrhythmias tachycardia and fibrillation We believe that quinidine and/or digitalis prior to the countershock is an important cause. We now recommend avoidance of these drugs until after the shock Another impressive feature in our series is the extremely low incidence of embolism Although difficult to prove our firm belief is that the consistently administered anticoagulation therapy may be an important factor

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CLINICAL AND METABOLIC EFFECTS OF DIFFERENT DOSES OF PROSTAGLANDIN E_1 IN MAN

Prostaglandin and Related Factors

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Abst act Prostaglandin E_1 (PGE_1) was infused intravenously into eight healthy male subjects The dose was successively increased to the maximal tolerable dose and varied from 0 03? to 0 58 $\mu g/kg/min$ When the lower doses were given there were either no clinical effects or only a flushing of the face When the dose was increased the flushing generally became more intensive and extended to other parts of the body mainly the arms and hands In some of the subjects the flushing d minished and even changed to pallor with the highest doses of PGE All subjects complained of headache which usually became severe In one of them the headache was unilateral and accompanied by visual symptoms All subjects developed cramps in the upper part of the abdomen which generally became so severe that the administration of PGE_1 had to be stopped Several of the subjects also felt sore in other parts of the body such as the back and legs. The various symptoms disappeared within 15–30 min after the end of the infusion

The arterial levels of free fatty acids in blood plasma increased in all subjects In several subjects the rise was discontinuous and in two of them a decrease in the level of FFA was seen. The FFA response to PGE_1 was neither related to administered dose nor to clinical effects Turnover rate studies with labelled palmitic acid showed that the plasma FFA changes were mainly caused by variations in the rate of mobilization of FFA into blood The plasma levels of glycerol showed the same type of response to PGE_1 as the FFA levels No significant changes in blood glucose were seen in the seven subjects who were fasting. In one subject who had had breakfast there was a rapid fall in the concentration of blood glucose

Prostaglandin E_1 (PGE_1) which has smooth muscle stimulating and vaso-dilatory activity was found to have some interesting clinical effects when infused into man (4) Two of three healthy subjects infused with PGE_1 at a rate of 0 1 $\mu g/kg/min$ for 20 minutes exhibited facial flushing and complained of headache In addition one of these subjects who did not suffer from migraine had visual symptoms in the form of lightnings and other colored phenomena

Furthermore PGE_1 has significant metabolic and cardiovascular effects PGE_1 inhibits basal as well as catecholamine stimulated lipolysis in adipose tissue from the fed rat and from man when these tissues are incubated in vitro (2 3 17 27 28) PGE_1 also inhibits the catecholamine induced stimulation of mobilization of free fatty acids (FFA) in vivo in the fasted dog (5 6 28)

However when PGE_1 was infused into fasting man a rise in plasma levels of FFA and glycerol was found suggesting a stimulation of FFA mobilization from adipose tissue (4) Further studies showed that in fasted dogs both anesthetized and non anesthetized infusion of PGE_1 might increase the FFA levels and stimulate the FFA mobilization (6) Continued work showed that the FFA response to PGE_1 in fasted dogs was dose dependent PGE_1 infused at lower rates increased the concentration of FFA in blood plasma while higher doses decreased it (7)

The present study was made to elucidate the frequency and the dose relationship of the various clinical effects of PGE_1 in healthy men and also to investigate whether the FFA response to PGE_1 is dose dependent in man in the same way as in the dog PGE_1 was infused i v into fasting male subjects and the dose successively increased every 30 minutes until the maximal tolerable dose was reached Clinical effects were recorded and arterial plasma levels of FFA and glycerol and blood glucose were followed The FFA turnover rate was also studied in some of the experiments

Table I *Clinical effects of PGE_1*

PGE_1 was infused i v at increasing doses for 30-min periods as indicated in Fig 1

Subject	μg PGE_1 infused i v per min and kg 0 032	0 056	0 10	0 18	0 32	0 58	After
1	0	F H	H F ↓ P C	H A T	H A V	—	20
2	0	0	F	F H	F H A	F H A ↓ P	15-25
3	—	—	F H	F H A Leg cramps	F H A	—	10-20
4	—	0	F H Vis	F H ↓ P C	H A N	—	Unilateral H 30-35
5	—	F	F H A N	—	—	—	20
6	—	F	F A	F H A D	—	—	20-25
7	—	T H F ↓ P	H A	—	—	—	30
8	—	F	F Pains in the back	F A N H ↓ P Pains in the back Restlessness	—	—	20-30

0 = no symptoms F = flush H = headache P = pallor C = cold T = tiredness A = abdominal cramps N = nausea V = vomiting Vis = visual symptoms D = dyspnea Bold letters indicate reason for finishing due to untolerable symptom

Figures under *After* indicate duration of symptoms in minutes

by means of a constant infusion of labelled palmitic acid

METHODS

Procedure

Eight male volunteers aged [illegible] to 31 years were studied They were healthy as judged from routine clinical and laboratory investigations Exercise tests did not show any abnormalities in working capacity ([illegible]) or ECG The heart volume and the total amount of hemoglobin were also determined in some of the subjects and the values were within normal limits

The subjects reported at the laboratory at 8 a m. after fasting overnight. One catheter of teflon was placed percutaneously into the brachial artery after local anesthesia with Carbocain® (Bofors) In the same arm a double lumen heart catheter was introduced with the end hole in the pulmonary artery The heart catheters were used for circulatory studies and the data obtained will be published separately (19) Short teflon catheters were introduced into the veins of the opposite arm for infusion of PGE_1 and labelled palmitic acid With all catheters in place the subjects rested comfortably in the supine position throughout the study

Prostaglandin E was obtained from Professor S Bergstrom as the crystalline preparation isolated from sheep prostate glands (8 10 11) PGE_1 dissolved in saline was sterilized by ultrafiltration This sterile solution, containing 50 μg/ml was dispensed in 5 ml portions and stored at − 15 C The solution was diluted in 0 9 per cent saline 2-5 times immediately before infusion

Analysis

Arterial blood was withdrawn into heparinized syringes. Aliquots of blood were precipitated for determination of glucose and the remainder was promptly centrifuged to separate cells from plasma The plasma was immediately processed and FFA determined according to Dole ([illegible]) with the modification described by Trout et al. (9) Plasma glycerol was determined by the enzymatic method of Wieland (30) and blood glucose according to Marks (25)

Turnover rate studies

The plasma FFA turnover rate was studied by infusing palmitate 9 10 H bound to human albumin (Kabi Stockholm, Sweden) at a constant rate ([illegible]) The procedure has been described in detail previously from this laboratory ([illegible] 23)

RESULTS

Clinical Effects

The clinical effects are summarised in Table I and a more detailed description of each study is given below

Subject 1 The PGE_1 infusion was started with 0 032 μg/kg/min and the dose successively increased up to 0 32 μg/kg/min During infusion of 0 032 μg of PGE_1 there were no symptoms When the dose was increased to 0 056 μg he complained of headache and his face flushed During administration of the following dose 0 1 μg the subject became pale and felt cold With 0 18 μg he complained of tiredness abdominal cramps and severe headache When 0 32 μg/kg/min of PGE_1 had been given for 5 min the subject vomited and the infusion was stopped All the symptoms had disappeared about 20 min later

Subject 2 With the first two doses 0 032 and 0 056 μg/kg/min there were no clinical effects When 0 1 μg had been given for about 15 min his face flushed During the administration of the following dose 0 18 μg the subject complained of headache and became redder With 0 32 μg he complained of abdominal cramps localized mainly to the upper part of the abdomen The face flushed and the hands were red and swollen When 0 58 μg/kg/min of PGE_1 had been given for 15 min the infusion was interrupted because of severe abdominal pains During the last 5–10 min of infusion the flushing of the face and hands diminished The subject felt normal 15–25 min after the end of the PGE_1 infusion

Subject 3 The PGE_1 infusion was started with 0 1 μg/kg/min After about 10–15 min the face reddened and the subject felt hot in his face About 10 min later he complained of a pulsating headache at the front During the infusion of 0 18 μg these symptoms became worse and there were also pains in the abdomen and in the hips and thighs The highest dose 0 32 μg/kg/min was given for only 12 min because of a severe headache The face still flushed when the infusion was stopped The pain in the legs and the headache lasted for 10–20 min after the end of infusion

Subject 4 During administration of the lowest dose 0 056 μg/kg/min there were no symptoms After infusion of 0 1 μg for 10 min the subject complained of a pressure above his front and flashes of light When 0 18 μg of PGE_1 had been infused for about 25 min he felt cold and his face was sweaty During administration of 0 32 μg/kg/min the headache became more intensive and after 15 min there were cramps in the upper part of the abdomen and nausea As these symptoms became worse no further dose was given About 20 min after the end of infusion the headache was unilateral (right side) After another 10–15 min all symptoms had disappeared

Subject 5 received only two doses 0 056 and 0 1 μg/kg/min during a 60 min period After 30 min of PGE_1 infusion the face flushed After 10 min with 0 1 μg of PGE_1 the subject complained of tensions everywhere in the body Ten min later he complained of a severe headache which was quite a new experience for him He also complained of abdominal pains and nausea and his face and ears were flushed All the symptoms disappeared 20–25 min after the end of infusion

Subject 6 The PGE_1 infusion was started with 0 056 μg/kg/min and after about 15 min the subject complained of itching in his cheeks Ten min later his hands became hot When the next dose of PGE_1 had been given for about 25 min there were pains in the upper part of the abdomen During the infusion of 0 18 μg of PGE_1 the subject rapidly developed severe headache and dizziness The infusion had to be stopped after 25 min as the pains in the abdomen became worse and also because the subject felt restless had cough and difficulty in breathing Five min after the end of the administration of PGE_1 the various symptoms had disappeared except for the abdominal pains and headache which persisted for another 20 min

Subject 7 When the lowest dose 0 056 μg/kg/min had been given for 13 min the subject complained of dizziness and somnolence Soon after he developed a headache and felt restless There was a transient flush in the face and at the end of the infusion of the lowest dose he looked pale and sweaty When the next dose 0 1 μg was given the subject complained of increasing abdominal pains headache and tensions everywhere in the body It was therefore not possible to increase the dose The abdominal pains and tensions disappeared within 10 min after the end of the PGE_1 administration There was only a slight headache after 15 min and the subject felt quite normal after 30 min

Subject 8 When 0 056 μg/kg/min of PGE_1 had been given for about 25 min the subject started to flush in the face and hands and to

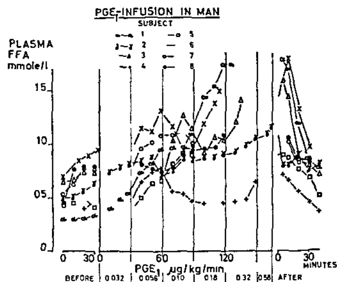


Fig 1 Effect of PGE on arterial plasma levels of FFA in eight male subjects PGE1 was given i v at a constant rate in doses which were successively increased as indicated in the figure The dose varied from 0 032 to 0 58 μg/kg/min.

sweat During administration of the next dose there were increasing pains in the back Five min after starting the highest dose the flush became less intensive and there were slight abdominal pains and nausea The pains in the back became more severe and the subject felt restless The infusion of PGE_1 was therefore stopped when the 30 min period with 0 18 μg/kg/min of PGE_1 had passed and the symptoms disappeared within 20–30 min

Metabolic Effects

The effect of PGE_1 on arterial plasma levels of FFA and glycerol varied considerably between the subjects *during* the infusion of PGE_1 as shown in Figs 1 and 2 *After* the infusion however the FFA level dropped in all subjects Due to the varying response as well as to the different clinical effects so that not all subjects could tolerate the same dose we do not feel that it is useful to calculate a mean FFA response curve from these data Instead the results will be briefly described for each subject in relation to the clinical effects

Subject 1 who had eaten in the morning before the study had the lowest initial FFA level which increased during the lowest infusion rate 0 032 μg/kg/min when no clinical effects occurred His FFA concentration continued to increase during all other rates of infusion

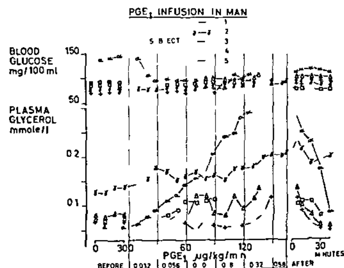


Fig 2 Effect of PGE on arterial plasma levels of glycerol and on blood glucose in five male subjects PGE_1 was given i v at a constant rate in doses which were successively increased as indicated in the figure The dose varied from 0 032 to 0 58 μg/kg/min

Subject 2 had a slightly increased FFA level before the infusion and this did not change significantly and no symptoms occurred with 0 032 μg and 0 056 μg During increasing doses up to 0 32 ug there was no major increase in FFA in spite of rather pronounced clinical effects During the later part of 0 32 μg and during 0 58 μg/kg/min there was a tendency towards an increase of FFA

In subject 3 FFA increased at 0 10 μg/kg/min (F H (abbreviations as in Table I)) remained fairly constant at 0 18 μg with increasing painful symptoms (F H A) and increased further with 0 32 μg of PGE_1

Subject 4 had raised FFA level with 0 056 μg/kg/min (no symptoms) At 0 10 μg his FFA fell and simultaneously symptoms appeared (F H Vis) The FFA level remained at a low level except in the last sample at 0 32 μg in spite of significant and painful clinical effects

FFA rose in *subject 5* with 0 056 μg/kg/min of PGE_1 (F) and continued to rise with 0 10 μg (F H A N)

Subject 6 had a continuous increase *before* the infusion so the increase seen with 0 056 μg (F) cannot be interpreted With 0 10 μg/kg/min of PGE_1 however FFA fell (F A) in order to increase with 0 18 μg (F H A D)

In *subject 7* FFA rose with 0 056 μg/kg/min (H F) and remained constant with 0 10 μg in spite of significant clinical effects (H A)

Subject 8 had unchanged arterial plasma FFA level at the infusion rate 0 056 μg/kg/min (F) and slight increase at 0 10 and 0 18 μg concomitant with pronounced clinical effects

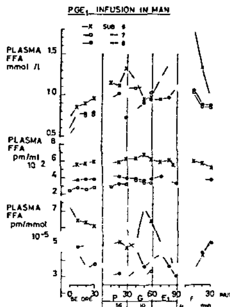


Fig 3 Effect of PGE on arterial levels of FFA and on activity and specific activity in the plasma FFA fraction in three male subjects PGE_1 was given i v at a constant rate in doses from 0 056 to 0 18 μg/kg/min as indicated in the figure A constant infusion of labelled albumin bound palmitate was given throughout the study (subj 6 1.98 subj 7 3 16 and subj 8 4 15 cpm × 10 /min)

It is evident that there was no correlation between clinical effects and FFA response The FFA level sometimes increased without symptoms (subjects 1 and 4) sometimes remained unchanged when flushing was the only clinical effect (subject 8) and also when there were pronounced clinical effects (subjects 2 3 7) This latter finding is striking as one would anticipate the FFA levels to rise when painful and annoying symptoms are present (14) There was even a decrease in FFA levels concomitant with rather pronounced painful and distressing symptoms in subjects 4 and 6 In all subjects there was sometimes an increase in the concentration of FFA during the study

The plasma levels of glycerol (Fig 2) either showed the same type of response to PGE_1 as the FFA this being most pronounced in subject 1 or remained almost unchanged After the infusion the concentration of glycerol in plasma decreased in all subjects

Turnover rate studies The activity in the isolated plasma FFA fraction (cpm/ml) was almost unchanged during the administration of PGE_1 (Fig 3) This indicates that the efflux of FFA from plasma (fractional turnover rate) was unchanged The specific activity in plasma FFA (cpm/mmole) decreased when the FFA concentration increased and increased when FFA levels fell These findings show that the changes in plasma FFA concentrations were mainly caused by changes in the rate of mobilization of FFA into the blood

Blood glucose There were no significant changes in blood glucose concentrations during administration of PGE_1 in subjects 2–5 In subject 1 (Fig 2) the blood glucose concentration was higher than in the other subjects before the administration of PGE_1 This subject admitted that he had had a meal in the morning a fact

also evident when he vomited It is of interest that his blood glucose levels fell rapidly during infusion of the lowest dose of PGE_1 when he reported no symptoms

DISCUSSION

The infusions of PGE_1 into man had various clinical effects Flushing of the face was seen in all subjects at an infusion rate of 0 1 ug/kg/min or less Infusions of prostaglandins E (9) and of PGE_1 (4) to man have previously been reported to cause facial flushing The mechanism of this effect is not known PGE_1 may have a direct vaso-dilatory effect on the vessels in the skin PGE_1 may also dilate the cutaneous vessels by releasing other vasoactive substances It is of interest that during infusion of serotonin into man a similar flushing as well as a rise in FFA concentration like that found during administration of PGE_1 can be seen (18) which may suggest a possible interrelationship between PGE_1 and serotonin Cabut and Vincenzi (16) recently reported that PGE_1 may disrupt mast cells in vitro If this mechanism also operates in vivo it may cause local or systemic release of serotonin The intense reddening and swelling of the left hand we observed during an intra arterial infusion of PGE_1 (4) may be pertinent That subject probably got the infusion directly into the left brachial artery

The infusions of PGE_1 induced a headache which was pulsating in all eight subjects In subject 4 the headache was temporarily unilateral He also complained of light flashes before the headache started Similar visual symptoms were described previously by another healthy person during infusion of PGE_1 (4) The symptoms were characteristic of migraine but none of the subjects had any history of this disease The headache and other migraine like symptoms may be related to a vasodilatory effect of PGE_1 or of other substances possibly released by PGE_1 The fact that the headache was accompanied by abdominal pain and nausea and occasionally by vomiting suggests that it would be interesting to study the effect of PGE_1 infusions in patients with migraine

Abdominal cramps of intermittent character occurred in all subjects It is likely that these symptoms were due to the well known smooth muscle stimulating effect of the prostaglandins which has been demonstrated in vitro on various isolated intestinal preparations It is of interest that the three major lung metabolites of PGE_1 have only about $^1/_{10}$ of the activity of PGE_1 in vitro on isolated intestinal smooth muscle preparation (31) This suggests that it was PGE_1 and not any metabolite of PGE_1 rapidly formed in the lungs that caused the abdominal cramps The flush always appeared before the abdominal symptoms the headache mostly so suggesting that higher doses of PGE_1 are needed for stimulation of intestinal smooth muscle than for obtaining vascular effects

In agreement with our previous results (4) infusion of PGE_1 increased the arterial plasma levels of FFA in fasting human subjects By turnover rate studies it was shown that the rise in FFA concentration was caused by an increased mobilization of FFA into blood and not by a reduced efflux of FFA from blood Furthermore when the FFA level fell in subject 6 this was due to an inhibition of the rate of FFA mobilization

Infusions of PGE_1 into fasting anesthetized as well as non anesthetized dogs may also increase the FFA mobilization (6 7) From our previous studies in dogs it was evident that the FFA response to PGE_1 was dose dependent (7) Lower doses of PGE_1 increased while higher doses decreased the plasma levels of FFA However in the present studies in man the FFA response was not related to the administered dose of PGE_1 In subjects 4 and 6 for example the FFA level was decreased at intermediate infusion rates In subject 6 this reduction was conclusively shown to be due to inhibition of mobilization of FFA but when the infusion rate was increased from 0 10 to 0 18 μg/kg/min the FFA level rose significantly due to enhanced mobilization Subjects 2 3 and 7 had unchanged FFA levels simultaneously with pronounced clinical effects and it is possible that this was a result of two opposite effects on mobilization a stimulation caused by the annoying symptoms (14) and an inhibition caused by PGE_1

In contrast to the continuous increase in plasma FFA levels seen during administration of noradrenaline into dog and man the FFA rise induced by the infusions of PGE_1 was often discontinuous or even transient Previous studies on dogs (6) have suggested that the effects of PGE_1 infusion on FFA mobilization are dependent on

at least two mechanisms a stimulation via the sympathetic nervous system seen during infusion of low doses and a direct inhibition of lipolysis in adipose tissue at high doses of PGE_1 The variable changes in plasma FFA concentration observed in man might be due to the operation of several different mechanisms such as effects on sympathetic nervous system changes in blood flow and inhibitory actions of PGE_1 in adipose tissue Infusion of PGE_1 to a patient with a traumatic central nervous system lesion with disturbed vaso-motor function suggesting an injury of the sympathetic nervous system did not cause any change in the levels of FFA and glycerol in arterial blood plasma (21)

The finding that PGE_1 may disrupt mast cells and release heparin (16) suggested that release of lipoprotein lipase might have occurred in man during infusion of PGE_1 We tested this by determination of lipoprotein lipase activity (12) in plasma samples obtained before and during infusion of PGE_1 (13) but there was no demonstrable effect

Studies on rat adipose tissue in vitro (3) demonstrated that the nutritional state is of importance for the effect of PGE_1 on FFA mobilization An inhibitory effect on the FFA release could be demonstrated only when the adipose tissue was taken from fed rats but not with adipose tissue from fasted rats Our previous studies (7) did not suggest that the nutritional state was of major importance for the FFA response in vivo in unanesthetized dogs In this study all subjects were fasting except one who had not followed the instructions In this subject PGE_1 elevated the plasma level of FFA and he had the most pronounced FFA increase This observation suggests that the nutritional state is not of importance for the FFA response to infusion of PGE_1 in man

Blood glucose levels did not change in the fasting subjects consistent with previous findings in dogs (6) and suggesting that there was no release of adrenaline or other hormones affecting glucose metabolism It is not known if the rapid fall in concentration of blood glucose from 140 to 100 mg/100 ml in the non fasting subject was due to enhanced efflux (peripheral or hepatic uptake) or from reduced entry (absorption) of glucose into the blood Nor do we know if this effect was due to hormonal or circulatory (splanchnic?) changes induced by PGE_1 We think that it is unlikely that it was due to a stimulation of insulin release in view of the pronounced increase in FFA levels

Neither the clinical effects nor the metabolic response to PGE_1 seemed to be related to the cardiovascular reactions (19) The cardiovascular response to PGE_1 indicated a positive chronotropic effect either acting directly on the myocardium or indirectly via the central nervous system There was also a marked decrease in systemic resistance which we think is mainly due to an opening of arteriovenous shunts In subjects 4 and 6 some of the symptoms during the infusion of the highest dose of PGE_1 might be due to a marked hyperventilation with alkalosis The possibility that pH changes observed during the PGE_1 infusions may affect FFA mobilization cannot be ruled out

It is an intriguing fact that PGE_1 has such a potent inhibitory action on lipolysis in vitro which cannot be consistently reproduced in man in vivo when PGE_1 is infused intravenously This lack of conformity between in vivo and in vitro results could be due to many mechanisms such as inactivation of PGE_1 in the lungs circulatory effects e g in adipose tissue activation of systems and hormones stimulating lipolysis etc The findings of this study do not rule out the possibility that PGE_1 plays a physiological role in FFA mobilization by acting in adipose tissue as a local tissue hormone modulating lipolysis e g by inhibiting the accumulation of cyclic 3 5 AMP (15) the probable activator of the triglyceride lipase (cf 15)

ACKNOWLEDGEMENTS

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THE MINERAL COMPOSITION OF SOME INDIAN AND INDONESIAN BLADDER STONES

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Abst act Examination of 70 bladder stones from India and Indonesia seems to show that a large number of them arise with a primary formation of a small ammonium hydrogen urate stone This is particularly pronounced in the bladder stones from boys 14 of 17 nuclei + Zone 1 being of this composition Further research on the etiology of these stones should thus be d ected toward the uric acid metabolism and excretion in children in the areas where the stones are endemic.

It has been shown that magnesium hydrogen phosphate trihydrate is of relatively frequent occurrence in these bladder stones and that this mineral has a characteristic location in relat on to magnesium ammonium phosphate hexahydrate

There is a marked excess of calcium oxalate monohydrate in relation to the dihydrate and apatite is found more rarely than in European stones.

In Asia a lithiasis of the urinary tract occurs which is designated as endemic urinary lithiasis It is characteristic of the disease that the stones are frequently localized to the bladder with a high prevalence in children especially boys and it is most commonly seen in poor rural districts The disease has been reported from several countries in Asia China (21) Thailand (16 23) India (1 4 13) Turkey (6) and Israel (10) It seems that the disease was previously spread over a larger area but it is believed that it is regressing as the standard of living improves (1 6)

Epidemiologic studies have been made in an attempt to discover the causes of the disease with special emphasis on nutrition because of its special socio-geographic distribution (1–3) The investigations have shown that the diet in the stone areas is deficient in animal protein and fat in vitamins A and C and carotene However no definite relations between these deficiencies and the development of stones have been demonstrated

Several investigations have been made of the chemical composition of these stones Thomsen from China (21) Newcomb and Ranganathan from India (15) and Eckstein from Turkey (6) found that urates were the most frequently occurring minerals in these stones Noble from Japan (16) and Andersen from India (1) found that calcium oxalate was the most common component All of these studies have been chemical not mineralogical and have taken no consideration of the structure of the stones Few mineralogical investigations have been published Epprecht and Schinz (7) examined ten stones from Mesopotamia with X ray diffraction and Parsons (17) examined six bladder stones from Nepal by the same method Ammonium hydrogen urate was found in these series a mineral seldom found in European stones today but common in a series of stones from Norfolk in England from 1770–1870 (11)

It is characteristic of bladder stones that they are built up of concentric layers around a nucleus (Fig 1) There is good reason to assume that the composition of the layers reflects the physiological state of the urine during the periods in which they are formed It would thus be important for investigating the etiology of the stones to identify the mineral composition of the individual layers It would be particularly interesting to determine the mineral composition of the nuclei of the stones since this probably reflects the condition at the time when their development began

It is generally recognized that even the smallest deposits in the urinary tract can lead to secondary deposits A qualitative investigation of the usual type or even a quantitative determination of the separate components of the stones would thus

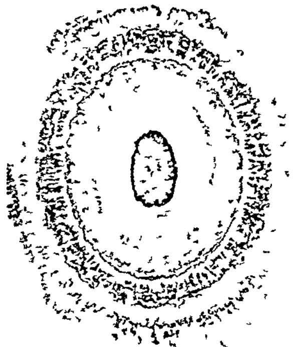


Fig 1 Section of a bladder stone

yield varying results according to the secondary conditions prevailing during the period of formation On such a total analysis even the contents of the nucleus would disappear in the larger mass of secondary deposits

On the basis of these considerations we have examined a number of bladder stones from India and Indonesia with the aim of identifying the minerals in the individual concentric layers and we have made special efforts to identify the minerals in the nuclei

METHOD

There are several methods for identifying minerals, but the most widely used in mineralogy is by using X ray diffraction. We have chosen this method for our investigation of stones in the urinary tract The principle involved is that a monochromatic X ray is spread when it passes through a crystal of unknown substance The direction of the resulting spread rays is determined by the distance between the various planes of the crystal and the intensity of these diffracted rays is determined to some extent by the individual subgroups within the more complicated molecules.

In practice a small amount of the sample is crushed with a pestle to a fine crystalline powder which is fastened to the tip of a glass capillary with vaseline The capillary is then placed in the center of a chamber in which a film has been placed around the periphery The powder on the capillary tip is then irradiated with monochromatic X ray light, and the film is exposed to the diffracted X rays After development the film exhibits a pattern of lines which is characteristic for the crystalline structure of the sample The minerals in the sample are identified by comparison with the diffraction patterns of pure known minerals

We have used a 90 mm cylindrical Debye Scherrer camera and Mn filtered iron radiation

The stones were cut across the middle with a small circular saw trying to preserve the nucleus which was not always possible The cut surface was viewed in a microscope and small samples were removed from the nucleus and the individual layers with a dental drill.

As we did not have the necessary equipment in our own laboratory to take the films we made them at the Geological Museum of the University of Oslo and we are extremely grateful for their generous assistance with the technical arrangement for making the X ray diffraction films We then identified the samples by comparing with reference films of known minerals

MATERIAL

Indian stones

The stones were received in response to a request made to centers in India during the years 1960–62 to complete a questionnaire regarding the incidence of bladder stones and to send specimens

Fifty specimens were received and 49 examinations are recorded The most consistent series was one of 12 stones in boys between the ages of three and ten years from one center numbers 29–40 These were sent by Dr M. G Whittier Sharanstaan Hospital Banswara Rajastaan

Another series of 12 was obtained from the Museum of the Miraj Medical Centre Maharashtra Western India by courtesy of Dr A Fletcher Jr but details of age and sex were not available

The remainder were received in ones or twos from various centers sometimes with and sometimes without clinical details Of the 49 stones examined 17 were in children (nos 1 2? 29–40 41 50) 16 were in adults (nos. 9-21 46 47 48) and in the remainder the age was not known

A series of 11 kidney stones in adults was received from Dr Karanjavalla and Dr Colabavalla both of Bombay

Indonesian stones

Forty stones were received from Professor L D Eerland who wrote "From 1926–27 I worked in Java and during that period I operated on many Javanese patients with bladder stones"

The weight of the stones varied from 4 3 to 184 g Twenty-one stones were examined crystallographically

RESULTS AND DISCUSSION

Table I shows the minerals found in our material Table II shows which minerals were found in the

Table I *Minerals (with code numbers) found in the present material*

Code nos	Chemical name	Chemical formula	Mineralogical name
2	Calcium oxalate monohydrate	$CaC_2O_4 \cdot H_2O$	Whewellite
3	Calcium oxalate dihydrate	$CaC_2O_4 \cdot 2H_2O$	Weddelite
4	Calcium hydrogen phosphate dihydrate	$CaHPO_4 \cdot 2H_2O$	Brushite
5	Tricalcium phosphate	$Ca_3(PO_4)_2$	Whitlockite
6	Basic calcium phosphate apatite	$Ca_{10}(PO_4)_6(OH)_2$	Hydroxy apatite
7	Magnesium ammonium phosphate hexahydrate	$MgNH_4PO_4 \cdot 6H_2O$	Struvite
8	Uric acid	$C_5H_4N_4O_3$	
9	Ammonium hydrogen urate	$NH_4C_5H_3N_4O_3$	
10	Sodium hydrogen urate	$NaC_5H_3N_4O_3$	
11	Magnesium hydrogen phosphate trihydrate	$MgHPO_4 \cdot 3H_2O$	Newberyite

nuclei and in the individual layers Table III shows the number of occasions on which the various minerals were demonstrated in the nuclei and the several layers

Ammonium hydrogen urate was found very often especially in the central parts of the stones It was found in 47% of all the nuclei examined and with decreasing frequency in the more peripheral zones There are however certain circumstances which make an accurate determination of the mineral content of the nuclei quite difficult The nuclei have a diameter of about 1–2 mm When the stones are cut some of the nuclei may be lost so that the first zone may be mistaken for the nucleus When samples are removed for the X ray diffraction procedure some of the adjacent zone may be included Both these sources of error lead to the most frequently occurring mineral in the first zone namely calcium oxalate monohydrate often being recorded as a constituent of the nucleus

With the stones from children Table II shows that 14 "nuclei + Zone 1 from 17 samples consisted of ammonium hydrogen urate Correspondingly in adult stones this mineral was found in nuclei + Zone 1 in only three of 16 stones

We believe that the results indicate that in the majority of the stones in children the origin was a small stone of ammonium hydrogen urate The primary cause of these stones must be sought in uric acid metabolism and excretion

Calcium oxalate monohydrate and calcium oxalate dihydrate The most frequently occurring mineral in the stones was calcium oxalate monohydrate which was found in 45–67 % of all nuclei and zones (Table III) It is remarkable that it is almost exclusively the monohydrate which was found In Western European and North American materials the dihydrate is found with about the same frequency as the monohydrate (5 8 9 14 18 20) There is disagreement as to whether monohydrate is a primary formation Tovborg Jensen (22) and Murphy and Pyrah (14) assume that there are inhibitors in the urine which prevent the precipitation of monohydrate As the monohydrate is found almost exclusively in Indian and Indonesian stones it may be that there are different stabilizing factors in the urine of the Indian and Indonesian patients as compared with Northern Europeans It is possible that a recrystallisation of primarily precipitated dihydrate occurs but Prien and Frondel (19) found no such transformation in stones stored up to five years so this is unlikely

It is impossible to choose between the alternatives of calcium oxalate precipitation as part of the primary process or as a secondary concretion around a foreign body of ammonium hydrogen urate

Uric acid was found relatively frequently but it was rare in the nuclei or Zone 1

Sodium hydrogen urate was found in only two stones

Magnesium ammonium phosphate hexahydrate was found rarely in the nuclei (3) and with a frequency of 10–20 % in the zones It is reasonable to assume that this mineral reflects infections during the growth of the stones

Magnesium hydrogen phosphate trihydrate is a

Table II *The composition of nuclei and zones*

The numbers refer to the corresponding minerals in Table I
C = children aged 3–10 A = adults

Stone no	Age group	Nucleus	Zone 1	Zone 2	Zone 3	Zone 4	Zone 5	Zone 6	Zone 7	Zone 8	Zone 9	Zone 10
Indian bladder stones												
1	C	2+9	2+	2	2							
2		9+2	2	2								
3		2	2	8	2	8						
4		2	8	8								
5			7	7	7							
6			7	7	7							
7			9	2	2							
8			7	7	7							
9	A		2	8+2	8	8						
10	A	2+3	2+	2+								
11	A		8	8	8	8	8	8				
12	A		2	2								
13	A	8?	8	8	8							
14	A	2+6?	2+9	2+6?	2+6?	2	6?					
15	A		2	8+2	2	2	2+9	2				
16	A		2	8	8							
17	A		7	Blank	2+6							
18	A		2	2	2+	2	2	2				
19	A	11+	11	11+	9	9+2	7+					
20	A		2	2	2							
21	A		8+7	8+?	8+?	8+?						
22	C		2+9	2+9	2+9	2+9	2+	2+11	2+11	8	7	11
24			2	2								
25			2+9	2	2+	7	2+	Blank	7			
26		2	2+7	2	Blank	6?						
27		2	2	2+	2	2						
28		9	2+9	2	7	7						
29	C	9+	2+9	2+9?	2							
30	C		9	9+	2+9	2						
31	C		9+2	9+2	9+2	2						
32	C		9+2	2+7	2+9?							
33	C	9	8+9?	8	8							
34	C		9	9+2	8							
35	C		9+3	9+3	3							
36	C		9+2	2+9	2+9							
37	C		8+9?	9+8	8	8						
38	C		2+9	2+3	2+							
39	C		7+2	2+7?	9+2	8						
40	C		2	2	5	4						
41	C	9+	2+9	2+9	8	9+	8	9				
42		2+9?	2+9?	2+6	5+6							
43		7	6+7?	11								
44		10+6+	2+10	2+10	2+10	2+10	2+6					
45		2+	9+8+									
46	A	9	9+2	2+9	2							
47	A	7	7	7								
48	A	2	9?	Blank	?	8						
49	C	2	2	2								
50	C	3+9	9+10	3+9	3+9							
Indonesian bladder stones												
1			2	2								
2		Blank	9+2	9+2?	2	2+3?						
3		Blank	9+	3	2+3							
4		9+?	2	2+9	2	7	11					
5		2+9	2+	2	7	11						
6		2+9?	7?	7+2	7	7+11?						
7			2+9?	2	7?	2						
8			2+3	2+3								
9			7	7								

Table II (*Co ti ed*)

Stone no	Age group	Nucleus	Zone 1	Zone 2	Zone 3	Zone 4	Zone 5	Zone 6	Zone 7	Zone 8	Zone 9	Zone 10
10			2	2	2	2						
11			7	7	7?	11						
12		2	2+	7	7							
13		2	2+7	7	7+11?	7+11?						
14			2+	7?	7+2							
15		2+9	2+	2	2							
16			9	2	2+							
17			2	2	2	2	2					
18		9+	9	2	2	7	7					
19		9+2	2+9	2	2							
20		2	2	2	2	2						
25			2	8	2	2						

Table III *Number of demonstrations of the individual minerals in nuclei and zones*

Mineral Code nos	*Nucleus* 3. samples	*Zone 1* 70 samples	*Zone 2* 67 samples	*Zone 3* 56 samples	*Zone 4* 33 samples	*Zone 5* 12 samples
2	18 (67)	44 (63)	43 (64)	32 (57)	15 (45)	6 (50)
3	1	2	5	3	1	
4					1	
5				2		
6	2		2	3	1	
7	2 (3)	12 (17)	12 (18)	11 (20)	4 (12)	1
8	1	7	11	9	7	2
9	15 (47)	26 (37 %)	13 (20)	8 (14)	3 (9)	1 (8)
10	1	2	1	1	1	
11	1	1	2	1	4	1

Table IV *The composition of some Indian kidney and ureter stones*

The numbers refer to the corresponding minerals in Table I

Stone no	Area 1	Area 2	Area 3	Area 4
2	2	2	4	
3	2	2+	Blank	2
4	2	2	2	
5	3			
6	2+	2		
7	2	2	2	Blank
11	8	2	8	
14	2	2	2	
15	2	2	3+2?	
16	2	2+	2	2
18	2			

mineral which *apparently has only been found* previously in three stones Parsons (17) found it in one stone together with apatite Herring (8) found it in one stone together with apatite in a series of 10 000 stones and Murphy and Pyrah (14) found it together with magnesium ammonium phosphate hexahydrate in one of 250 stones In our small series of 70 bladder stones we have found it in eight In most cases as seen in Table II it was found outside a zone of magnesium ammonium phosphate hexahydrate This location might indicate that the hydrogen phosphate tri hydrate is formed by a recrystallisation of the ammonium phosphate with a loss of NH_3 But as the hydrogen phosphate trihydrate is so rare in European stones this explanation is unlikely It is more probable that the hydrogen phosphate trihydrate is also a primary precipitation Parsons and Herring s two stones in which the hydrogen phosphate was found along with apatite also support this assumption

Apatite is very frequent in European stones (for references see under calcium oxalate) In comparison there was remarkably little apatite in our material of Indian and Indonesian stones

Calcium hydrogen phosphate dihydrate was found in one stone outside a layer of tricalcium phosphate

Tricalcium phosphate was found in two stones in one with calcium hydrogen phosphate di hydrate in the other with apatite

A small number of kidney and ureter stones from India were also included in our investiga tion and the results are shown in Table IV Here also there was the same tendency to an excess of calcium oxalate *monohydrate*

Addendum After this manuscript was prepared Lonsdale et al (12) published the finding of magnesium hydrogen phosphate trihydrate in ancient English stones and in stones from Thailand and Turkey

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COUMARIN ANTICOAGULANT REQUIREMENT IN RELATION TO SERUM CHOLESTEROL AND TRIGLYCERIDE LEVEL

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Abstract The relationship of warfarin requirement to serum cholesterol and triglyceride level was studied in 91 patients who were on a well stabilized long term anticoagulant therapy In male patients a significant positive correlation was observed between warfarin requirement and serum cholesterol level A similar trend was observed in female patients although the correlation did not reach statistical significance No correlation was present between warfarin requirement and serum triglyceride level

The concentration of warfarin in the plasma 14–15 hours after the preceding warfarin dose was determined in the 91 patients on long term warfarin therapy The plasma warfarin level varied from 0.5 to 6.2 mg per liter with a median value of 2.0 mg per liter When a serum cholesterol value of 300 mg/100 ml was chosen as a dividing line between normocholesteremia and hypercholesteremia, the median "therapeutic" plasma warfarin level was found to be somewhat higher in hypercholesteremic patients than in normocholesteremic patients

The wide range of individual variation in the "therapeutic" plasma warfarin level indicates that, in addition to the individual variation in the rate of warfarin metabolism there are considerable individual differences acting at the level of the receptor site for coumarin drugs

The plasma warfarin half life was determined in 37 subjects. The mean half-life of warfarin was 35.6 hours (S.D. 19.1 hours) The range of individual variation was from 10 to 90 hours. There was no correlation between the half life of warfarin and serum cholesterol or triglyceride level

The duration of the depression of the prothrombin complex clotting factors after a single warfarin dose was shorter in hypercholesteremic than in normocholesteremic subjects when related to the half life of warfarin in the plasma A possible explanation of this phenomenon is that a greater amount of vitamin K is available at the receptor site for coumarin drugs in hypercholesteremic subjects. The results support the hypothesis that hypercholesteremia may be associated with increased levels of vitamin K in the plasma and liver

The dosage of coumarin anticoagulant drugs required to achieve a therapeutic depression of prothrombin complex clotting factors shows great individual variation The rate of metabolism of a given coumarin drug is known to show about ten fold variation in different individuals but to be remarkably constant in each individual (3 12 13 25) Individual differences in the affinity of the receptor site for coumarin drugs in the liver cells may also be partly responsible for the variation in the anticoagulant response (21) Furthermore the response to coumarin drugs is dependent on the rate of clotting factor synthesis in the liver cells This determinant of the coumarin response is influenced inter alia by the amount of vitamin K available in the diet (22 23) Anticoagulant tolerance may be decreased due to impairment of clotting factor synthesis in liver disease (25) Simultaneous therapy with some other drugs may alter the patient's response to coumarin anticoagulants either by interfering with the drug metabolism by altering the affinity of receptor site for coumarin drugs or by directly interfering with the synthesis of the clotting factors (e g 1 2 6 17 19 20)

When supervising long term anticoagulant therapy of patients with various cardiovascular disorders we as obviously also others have observed that patients with hyperlipidemia especially with hypercholesteremia often require high doses of coumarin or indanedione drugs for the maintenance of therapeutic anticoagulation In experiments on rats it has been found also that hyperlipidemia induced by feeding high fat diets either alone or in combination with thiouracil renders the animals resistant to the effect of coumarin or indanedione anticoagulants (5 18 26)

In this investigation the relation of warfarin

requirement to serum cholesterol and triglyceride level was studied in patients receiving long term anticoagulant therapy The plasma concentration of warfarin was also determined in these patients The half life of warfarin in the plasma was estimated in healthy volunteers and in patients showing varying degrees of hyperlipidemia Finally the relationship between the half life of warfarin and the duration of the clotting factor response after a single dose of warfarin was compared in normocholesteremic and hyper cholesteremic subjects

MATERIAL AND METHODS

A total of 91 patients 62 men and 29 women receiving long term anticoagulant therapy with warfarin (warfarin sodium Marevan®) were studied The mean age for the whole series was 53 9 (range 37–76)—52 7 for males (range 37–74) and 56 6 for females (range 38–76) The indication for anticoagulant therapy was coronary heart dise se in 6_ cases cerebrovascular disease in eight other obstructive arterial disease in seven rheumatic valvular heart disease with atrial fibrillation in nine and deep leg venous thrombosis in five cases The patients were free from known liver kidney or gastrointestinal disease and none of them had congestive heart failure Before and during the study the patients did not receive any drugs known to affect serum lipid levels or anticoagulant response The PP (prothrombin plus proconvertin) method of Owren and Aas (14) was used in the control of anticoagulant therapy All the patients included in the study were on a well stabilized anticoagulant treatment The PP levels at the time of the study were within the range of 7 to _5 per cent

The patients attended the out patient clinic in fasting state between 8 and 9 a m Blood samples were taken for the estimation of serum cholesterol by the method of Pearson et al (15) and serum triglycerides by a modification of the methods presented by van Handel and Zilversmit (?4) and Carlson and Wadstrom (4) as described by Pelkonen (16) Blood samples mixed in the proportion 9 1 with 3 8 per cent sodium citrate were collected in siliconized glass tubes for determination of the PP level and estimation of the warfarin concentration in the plasma according to the method described by O Reilly et al (11) Since individual plasma blanks could not be obtained from patients on long term anticoagulant therapy the average net optical density obtained from 30 plasma samples was used in the calculation of plasma warfarin concentrations

The patients had been instructed to take their daily warfarin dose at 6 p m. The blood samples were thus collected 14–15 hours after the preceding warfarin dose The average weekly requirement of warfarin (mg/kg) was calculated from the dosage of warfarin during a two-week period—one week before and one week after the collection of blood samples

The half life of warfarin in the plasma was determined

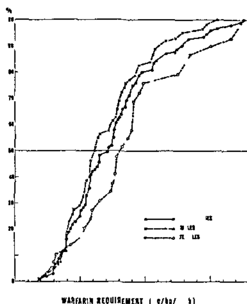


Fig 1 Cumulative frequency distribution of the whole material and male and female patients separately according to warfarin requirement

in 12 healthy volunteers aged ?3 to 35 and in ?5 patients aged 23 to 70 A standard dose of warfarin, 0 7 mg/kg of body weight was given in the morning to the subjects who had fasted overnight The drug was administered orally to 30 subjects and intravenously to seven Before the test dose blood samples were taken for determination of serum lipids and PP level as well as for estimation of the plasma blank used in the plasma warfarin determination Thereafter blood samples for warfarin and PP assay were taken 12 24 36 72 and if possible 1?0 and 144 or 168 hours after the administration of warfarin The half life of warfarin in the plasma was determined from the exponential part of the disappearance curve plotted on a semilog scale

RESULTS

Distribution of warfarin requirement

Fig 1 shows the cumulative frequency distribution of warfarin requirement in the 91 patients The median weekly requirement of warfarin in the whole series was 0 72 mg/kg and 97 5 per cent limits of the distribution were 0 29 and 1 67 mg/kg The median value for males was 0 62 mg/kg and for females slightly but not significantly higher 0 81 mg/kg The warfarin requirement showed no correlation to age

Warfarin requirement in relation to serum cholesterol and triglyceride level

There was a significant positive correlation between warfarin requirement and serum cholesterol

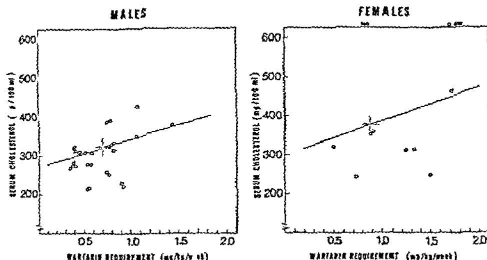


Fig 2 Relationship of warfarin requirement to serum cholesterol level in male and female patients Regression equation for males $y = 269.1 + 74.8\ x \pm 64.4$ $r = 0.34$ ($p <$ 0.01) Regression equation for females $y = 300.0 + 85\ x \pm 112.3$ $r = 0.31$ ($0.0 > p > 0.10$)

level in males (Fig 2) A similar trend was observed in females although the correlation did not reach statistical significance There was no correlation between warfarin requirement and serum triglyceride level (Fig 3)

Plasma level of warfarin during long term therapy and its relation to serum cholesterol level

Fig 4 shows the alterations of plasma warfarin level during two successive days in a patient who took a daily maintenance dose of 5 mg of warfarin at 9 a m In the present study the patients were instructed to take their daily warfarin doses at 6 p m and the blood samples for the determination of the plasma warfarin level were taken 14–15 hours after the preceding warfarin dose Fig 4 indicates that this time falls on the exponential part of the disappearance curve following the administration of each warfarin dose As shown in Fig 4 PP level remains virtually unchanged throughout the day in spite of considerable alterations of the plasma warfarin level

Fig 5 shows the distribution of the plasma

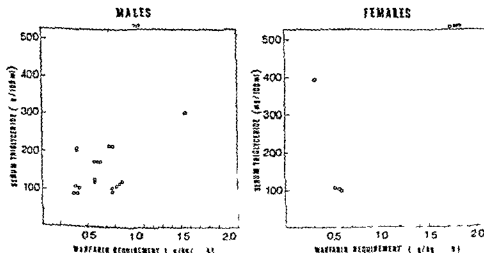


Fig 3 The absence of correlation between warfarin requirement and serum triglyceride level

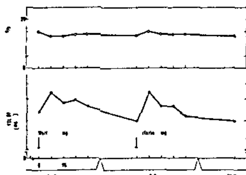


Fig 4 Alterations of plasma warfarin level and PP level during two successive days in a patient who took daily maintenance doses of 5 mg of warfarin at 9 a m The half life of warfarin in the plasma determined on a separate occasion was 40 hours

warfarin levels estimated 14–15 hours after the preceding warfarin dose in the 91 patients receiving long term therapy The range of variation was wide from 0 5 to 6 2 mg/l The median plasma warfarin level was 2 0 mg/l and the 95 per cent limits of the distribution were 0 7 and 3 5 mg/l In Fig 6 plasma warfarin levels are presented in relation to weekly warfarin requirements This figure also includes lines indicating the calculated relationship between the plasma warfarin level and anticoagulant requirement at various rates of warfarin metabolism These calculations are made on the presumption that orally administered warfarin is completely absorbed and that the average volume of distribution of warfarin is 13 per cent of body weight (13) The relationship between the plasma warfarin level and weekly warfarin requirement fits rather well

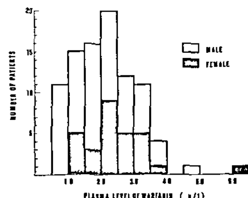


Fig 5 The distribution of the plasma warfarin levels determined 14–15 hours after the preceding warfarin dose in 91 patients on long term warfarin therapy

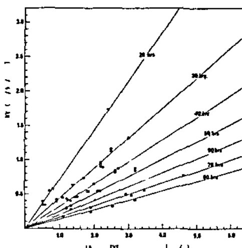


Fig 6 Plasma warfarin levels determined 14–15 hours after the preceding warfarin dose in relation to warfarin requirement The relationships between plasma warfarin level and warfarin requirement at different half lives of warfarin are indicated by calculated regression lines Open dots are values for patients with a serum cholesterol level < 300 mg/100 ml and solid dots for those with a serum cholesterol level ≥ 300 mg/100 ml

within the lines corresponding to warfarin half lives of 20 to 80 hours The distribution of the half lives of warfarin estimated in this indirect way corresponds rather well to that obtained by direct measurements (Fig 8) In three patients in the material presented in Fig 6 a direct measurement of the half life of warfarin made on a later occasion gave results close to those read from Fig 6 In Fig 6 as well as in later figures a serum cholesterol level of 300 mg/100 ml was arbitrarily chosen as a dividing line in classifying the patients as normocholesteremic or hypercholesteremic The most important point indicated by Fig 6 is that patients with the same half life of warfarin may show widely varying therapeutic plasma warfarin levels

The median therapeutic plasma warfarin level was somewhat higher in hypercholesteremic patients than in normocholesteremic patients although the difference was not statistically significant (Fig 7) The highest plasma warfarin levels were observed in hypercholesteremic patients

Plasma warfarin half life in relation to serum cholesterol and triglyceride level

After an initial mixing period the plasma warfarin concentration declined exponentially The

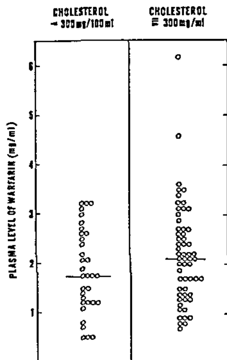


Fig 7 Plasma warfarin levels determined 14–15 hours after the preceding warfarin dose in normocholesteremic and hypercholesteremic subjects Median values are indicated by horizontal lines

half life of warfarin in the plasma was determined in 37 subjects It ranged from 10 to 90 hours The mean value was 35 6 hours (S D 19 1 hours) As shown in Fig 8 there was no correlation between the half life of warfarin and serum

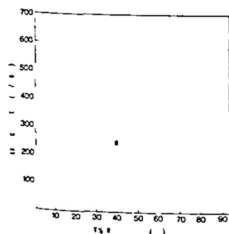


Fig 8 The absence of correlation between the half life of warfarin in the plasma and serum cholesterol level

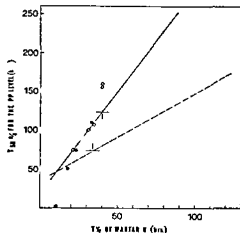


Fig 9 The relationship between the half life of warfarin in the plasma and the duration of clotting factor depression. Regression equation for subjects with a serum cholesterol level <300 mg/100 ml (open dots solid line) $y=19.0+2.64\ x \pm 49.1$ $r=0.63$ ($p<0.01$) Regression equation for subjects with a serum cholesterol level ≥ 300 mg 100 ml (solid dots dotted line) $y=35.7+1.1\ x \pm 33.0$ $r=0.64$ ($p<0.05$)

cholesterol level Neither was there any correlation between the half life of warfarin and serum triglyceride level

The correlation between the plasma warfarin half life and the duration of clotting factor depression in normocholesteremic and hypercholesteremic subjects

The duration of clotting factor depression induced by the warfarin dose of 0 7 mg/kg of body weight was estimated as $T_{50\%}$ for the PP level which is the time required for the PP level to attain the 50 per cent level during the restoration of clotting factors This time interval was chosen because its determination is more accurate than determination of the time in which the initial PP level is reached As shown in Fig 9 both in normocholesteremic and hypercholesteremic subjects there is a significant positive correlation between the plasma warfarin half life and the duration of the clotting factor depression The slopes of the regression lines in the two groups however differed significantly ($p<0.05$) In the hypercholesteremic group the restoration of the PP level occurred more rapidly than in the normocholesteremic group

DISCUSSION

The results obtained in this study have confirmed the clinical impression that the mean coumarin anticoagulant requirement is increased in patients with elevated serum cholesterol level On the other hand the serum triglyceride concentration seems not to influence the warfarin dosage

The daily dose of an anticoagulant needed for a depression of plasma clotting factors to a thera peutic level is extremely variable and apparently determined by a number of factors In view of this large individual variability demonstration of a dependence of the effective anticoagulant dose on any single factor is easily obscured by other variables Therefore the correlation revealed in this study between warfarin dosage and serum cholesterol concentration may be fairly strong be cause it becomes evident in spite of the wide scatter of individual warfarin requirements at each serum cholesterol level

The biochemical mechanism responsible for the interaction of serum cholesterol with the anticoagulant effectiveness is not clarified by the present experiments However the finding that the disappearance rate of plasma warfarin seems to be uninfluenced by serum cholesterol leaves the possibility that some inhibition of the drug action must occur at the level of clotting factor synthesis This view is compatible with the de monstration of slightly higher effective plasma warfarin concentrations in hypercholesteremic than in normocholesteremic subjects One of the possible factors operating at the receptor site of coumarin drugs is the amount of vitamin K Some years ago a hypothesis was presented by Nikkila and Pelkonen (9 10) that in hyperlipide mia especially in hypercholesteremia the level of vitamin K in the plasma and liver may be in creased So far suitable biochemical methods have not been available for the estimation of the small quantities of vitamin K in the plasma and other biological materials A close correlation has been demonstrated between plasma tocopherol and cholesterol levels (9 10) and the structural similarity between tocopherol and vitamin K sug gests the possibility that hypervitaminemia K is also present in hypercholesteremic subjects The finding that the duration of clotting factor de pression induced by a single warfarin dose when related to the half life of the drug is shorter in hypercholesteremic than in normocholesteremic subjects might be explained by a greater amount of vitamin K available at the receptor site for coumarin drugs in hypercholesteremic subjects The results of the present study thus support the hypothesis that hypercholesteremia may be as sociated with increased levels of vitamin K in the plasma and liver and that this is the factor to which the increased anticoagulant requirement is attributed

In the present material the median warfarin requirement was greater in females than in males which might be due to higher serum cholesterol levels in females On the contrary Merskey and Drapkin (8) have reported that anticoagulant re quirements on an average are greater in males than in females and that anticoagulant require ments decrease in both sexes with age In the present series warfarin requirement did not show a significant correlation to age but this lack of correlation may be explained by the small num ber of patients in older age groups

The mean half life of warfarin in the present series 35 6 hours (S D 19 1 hours) was some what shorter than the 44 hours reported by Ag geler and O Reilly (1) In the present series the range of individual variation of the half life of warfarin was from 10 to 90 hours and the range of variation reported by Aggeler and O Reilly was from 15 to 60 hours The shortest half life of warfarin so far observed in human is 6 5 hours (7)

Aggeler and O Reilly (1) determined the plasma warfarin levels in 12 patients receiving long term therapy Plasma warfarin levels determined 24 hours after the preceding warfarin dose varied in these patients from 1 to 5 mg per liter In the present study the plasma warfarin level was meas ured at a different time 14–15 hours after the preceding drug dose In spite of this the plasma warfarin levels observed—median 2 mg per liter and 95 per cent limits of the distribution 0 7 and 3 7 mg per liter—were lower than those observed by Aggeler and O Reilly In the patients studied by Aggeler and O Reilly one stage prothrombin time was used in the control of anticoagulant therapy It is known that the use of this method at the generally employed therapeutic levels re sults in a more intensive anticoagulation than with the PP method (8) This difference in the accomplishment of the anticoagulant therapy may

explain the difference in the therapeutic plasma warfarin level between the present series and that of Aggeler and O Reilly The difference in the effective plasma warfarin level in the two series may also at least partly be due to a dif ference in vitamin K content of an average American and Finnish diet There is no doubt that green vegetables and fruit rich in vitamin K are consumed much less in Finland than in the USA

ACKNOWLEDGEMENTS

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THE SIGNIFICANCE OF WORK LOAD AND INJECTED VOLUME IN XENON[133] MEASUREMENT OF MUSCULAR BLOOD FLOW

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Abstract Measuring peak flow in the anterior tibial muscle with radioactive xenon in normal and diabetic persons, it was shown that the same results were ob tained during ischaemia both after one minute of work and after maximal work.

There was no d fference when the peak flow was measured after the injection of 0 05 and 0 2 ml of the solution of rad oactive xenon

The coefficient of variation in repeated experiment was 18% for the peak flow and 30 for the latency time

In a group of non-diabetics (age range between 20 and 49 years) the resting flow was 1 6 ml per 100 g per min ±0 8 The peak flow after maximal work during ischaemia was 71 ml per 100 g per min±18 The mean latency time was 0 47 min±0 18

The local clearance method was first described by Kety (3) in 1949 The tracer used was radio active sodium and the intention was to study the circulation in the muscles Lassen et al (6) who replac d radioactive sodium by radioactive xenon demonstrated the many advantages of this tracer over radioactive sodium (6 7) and they need not be rep ated here

The following is a short report on some of the methodological studies of the local clearance method using radioactive xenon which were con sidered necessary as a preliminary study of the circulation in diabetics

METHODS AND MATERIAL

0 05 ml to 0.2 ml of a sterile isotonic solution of radio-active xenon (about 1 mC/ml The Radiochemical Centre Amersham) was injected into the anterior tibial muscle about 7 cm below the lower border of the tubercle of tibia and about 2 cm lateral to the anterior border of tibia. The injection was made with a sharp needle with an outer d ameter of 0 4 mm at a depth of 2 cm and an angle between the needle and the skin of 45 degrees The disappearance rate of the tracer was meas ured with a thallium activated NaI crystal coupled to a ratemeter The time constant was 3 sec The output from the ratemeter was recorded on a logarithmic po-tentiometer with a paper speed of 1 inch per min. The crystal was placed about 10 cm from the skin and the initial counting rate was generally between 100 000 and 300 000 counts per min

The subjects studied diabetics and non diabetics with an age range from 20 years to 63 years were at rest at least 15 min before the injection was given Measure ments of the resting clearance was started 5 min after the injection and was continued for at least 5 min Thereafter the foot was placed in a spe ially constructed ergograph and a cuff was placed around the distal part of the thigh The cuff was inflated rapid y from an air reservoir to a pressure of about 300 mm Hg. The pressure was maintained for 4 min and then suddenly released The ergograph was so constructed that a load of 2 kg was raised 12 cm when the foot was maximally dorsiflected and the load was supported when the calf mu cles were at rest The working speed which was controlled by a metronome was 50 contractions per min The patient started to work as soon as the cuff was inflated All studies were performed on males and the right anterior muscle was always used

The following problems were investigated

1 The relationship between the peak flow and the work performed was stud ed in 22 patients Every patient served as hi own control. In one experiment maximal work was performed i e until rendered impossible by pain In another experiment the patient worked for one minute Both examinations were performed with n a few days About half of the patients performed maximal work at the first examination

2 The relationsh p between the volume injected and the flow was stud ed in 16 patients Again e ery patient served as his own control and recei ed at the first exa mination 0 05 ml solution and at the second examina tion 0 2 ml or ice versa. All the patients performed maximal work

3 The reproducib l ty of the method was studied in an additional 9 pat ents Two examinations were per formed w thin a few days. All the patients performed max mal work

Calculations

The local xenon clearance method is based on the Fick principle The theoretical background and the principles behind the calculations have been presented many times before (3 4 5 6)

The fundamental assumption is that a complete equilibrium of xenon is maintained between muscles and blood As xenon is lost in the lungs and therefore does not recirculate the blood flow can be calculated according to the following simple equation

$$\text{flow (ml per 100 g per min)} = \frac{100\,\lambda\,k}{0\,4342} = 161\,k$$

where λ is the partition coefficient between muscle and blood (which according to Conn (1) is 0 7) k the fraction of one decade by which the tangent to the logarithmic curve of the counting rate decreased in one minute and 0 4342 is the correction for the logarithmic system used

Three parameters were calculated from the clearance curves 1 resting blood flow 2 peak flow after ischaemic work and 3 latency time or the period from release of the cuff pressure until the blood flow reached its maximal value

Conventional probability levels of significance have been used in the statistical analysis a p value greater than 0 05 not being considered significant

RESULTS

The relationship between the ischaemic work performed and the peak flow

Table I shows the results from 44 examinations in 22 patients The mean maximal work was 91 contractions and the peak flow averaged 58 ml per 100 g per min When the same patients performed 50 contractions (1 minute s work) the peak flow averaged 62 ml per 100 g per min This difference is not significant Neither was it possible to establish a significant difference when the patients were divided in diabetics ($n = 10$) and non diabetics ($n = 12$) There was no association between peak flow and the number of contractions performed during maximal work

Table I *The peak flow (ml/100 g/min) after maximal ischaemic work and ischaemic work during one minute*

Maximal work		Work during 1 min	
Peak flow ±S.D	Contractions performed ±S D	Peak flow ±S D	Contractions performed
58±17 n=22	109±21 n=22	62±20 n=22	50 n=22

Table II *The relationship between the injected volume and the maximal blood flow (ml/100 g/min)*

Volume injected		Volume injected	
0 05 ml	0 2 ml	0 05 ml	0 2 ml
85	78	59	55
70	53	38	40
86	93	46	63
50	74	43	48
70	57	49	24
50	56	70	74
30	27	61	58
110	107	62	61
69	73		

It is obvious that the work performed during one minute represents different fractions of the total working capacity in the single patient On the average the one minute work represented 55% ±13 of the total working capacity Calculations show that there is no association between the fraction of the total working capacity represented by the work during one minute and the peak flow measured after ischaemic work It must be concluded that the maximal stimulus to post ischaemic work flow as measured in these experiments is obtained by considerably less than maximal work

The relationship between the injected volume and the flow

The results are given in Table II There was no significant difference between the means of the two peak flows

Reproducibility

The reproducibility of the peak flow was calculated from 29 paired observations The mean flow was 60 ml per 100 g per min (range 27–110 ml) the standard deviation ±11 and the coefficient of variation 18% The reproducibility of the latency time was calculated from 29 paired observations The mean latency time was 0 37 min The standard deviation was ±0 11 and the coefficient of variation 30 %

Normal values

Blood flow was measured in 41 non-diabetics. None of them had symptoms or signs of peripheral vascular disease The ages ranged between 20 and 49 years mean 33 years The mean

resting flow was 1 6 ml per 100 g per min ±0 8 The mean peak flow was 71 ml per 100 g per min ±18 No association could be demonstrated be twe n the maximal blood flow and age The mean latency time was 0 47 min±0 18

DISCUSSION

Earlier measurements of the peak flow after ischaemic work by venous occlusion plethysmo graphy (2 10) have suggested that the peak flow increases as the ischaemic work increases Al thou h this is true for shorter periods of work the resul s presented here show that there is no difference between the peak flow after one minute of work during ischaemia which represents 55 % of th maximal work that could be performed and the peak flow after maximal ischaemic work The results are in accordance with those presented by Lindbjerg (9)

Thi fact is of course of great importance when comparing peak flows in persons with dif ferent work capacities

There are no quantitative data in the literature about the relationship between the maximal blood flow and the volume injected From the results obtained here it is obvious that the peak flow is the same whether 0 05 ml or 0 2 ml is used showing that the exact amount injected is not critical for evaluation of flow

Range and mean values for maximal blood flow latency time as well as experimental reproducibility are of the same order of magni tude as those obtained by Lindbjerg (8)

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MUSCLE BLOOD FLOW MEASURED BY XENON[133] AND VASCULAR CALCIFICATIONS IN DIABETICS

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Abstract A study of blood flow in the anterior tibial muscle was performed in a series of 75 young and middle aged diabetics Only three of the patients had symptoms and signs of peripheral vascular insufficiency in the legs

Peak muscular flow after maximal work during ischaemia was reduced in the diabetic patients and decreased with increasing duration of diabetes

Medial calcification of the arteries was common and it was possible to demonstrate that the decrease in maximal blood flow with increasing duration of diabetes was associated with the presence of arterial calcification

The latency of peak flow was reduced in the diabetics as has been previously observed by other authors

The increased frequency of *vascular abnormalities* in the lower extremities of diabetics is well established from the histological the roentgenological and the functional points of view However in younger diabetics *clinical vascular disease* is not seen very frequently Earlier studies of the vascular function in the lower extremities have concentrated on the blood flow in the skin but as will be discussed elsewhere (2) the results have been conflicting There are few measurements of muscular blood flow in diabetics The main purpose of the present study was to elucidate the following problems 1 Is the blood flow in the muscles abnormal in diabetics and particularly in younger diabetics? 2 Does it decrease with increasing duration of the disease?

METHODS

Blood flow was measured in the right anterior tibial muscle by radioactive xenon at rest and after maximal ischaemic exercise A methodological study of this procedure has been presented elsewhere (3)

A quantitative estimate of nervous function was obtained by measuring vibratory perception threshold in the big toe Vibratory perception threshold was measured with a biothesiometer (Bio-Medical Instrument, Ohio) and *expressed in volts In addition the patellar and achilles* tendon reflexes were examined in all patients Other procedures included palpation of the dorsal pedal artery and the posterior tibial artery measurements of the blood pressure before and after ischaemic exercise measurements of the subcutaneous fat thickness at the injection site and estimation of the haemoglobin concentration of the blood

X ray films were taken of the thigh the knee and the foot with the specific purpose of demonstrating arterial calcification An anterior posterior film was taken of the thigh a lateral film of the knee Dorsal plantar and lateral films were taken of the foot At the level of the thigh and the knee the radiologically demonstrable arterial calcifications could easily be divided into medial calcification and intimal calcificaton (10) X ray films were also taken of the calf but it was difficult to get a clear picture of the calcification presented here and the results from this examination will not be discussed

Conventional probability levels of significance have been used in the statistical analysis a p value greater than 0 05 not being considered significant

MATERIAL

Seventy-eight men were selected at random from the out patient clinic without knowledge of their vascular and nervous state The age ranged from 19 50 years with a mean age of 34 years The mean duration of diabetes was 14 years (range 0–35 years) Three patients were rejected from the study one patient was not able to work *for one minute during ischaemia (3) one patient* had gross oedema of the legs and one patient was not examined adequately The material thus included 75 patients

Results from measurements of blood flow with the same technique in a group of 41 non-diabetic men (age range 0–49 years, mean age 33 years) have been presented elsewhere (3) For comparison roentgenological examination of the arteries in the lower extremities was also performed in a group of 25 non-diabetic men (age range 1–49 years, mean age 37 years)

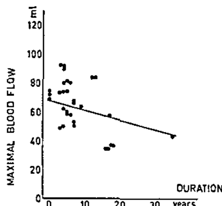


Fig 1 The relationship between maximal blood flow and duration of diabetes

RESULTS

Blood flow in the anterior tibial muscle

The mean resting flow was 1.8 ± 1.0 ml per 100 g per min. No association could be demonstrated between the duration of diabetes and resting blood flow. Fig 1 shows the relationship between the maximal blood flow after excercise during ischaemia and the duration of the disease. It appears that the maximal blood flow decreases with increasing duration of disease. Regression analysis was performed with age and duration of disease as the invariables. A significant relationship could be demonstrated between duration of disease and maximal blood flow ($p < 0.005$). No association could be demonstrated with age. The equation of the regression line is

$$y \text{ (ml per 100 g per min)} = -0.662\, x_{(\text{duration})} + 68$$

The standard error of the regression coefficient is 0.225. Mean maximal blood flow in the diabetics was 60 ml per 100 g per min ± 21. In the non diabetic group (3) the mean flow was 71 ml per 100 g per min ± 18. The two means differed significantly ($p < 0.01$).

Arterial calcification

X ray films were available in 71 of the 75 diabetic men. Twenty two patients or 31% showed calcifications. Of the 25 non-diabetics only one showed calcifications. This difference is significant ($p < 0.02$). Four of the 37 patients with a duration of diabetes less than ten years and 18 out of 34 with a duration of diabetes more than ten years showed calcifications. This difference is significant ($p < 0.001$). The mean age was however a little lower in the group of patients with the shortest duration of diabetes (32 against 36 years). Considering only those who were 30 or more years of age *there were four of 20 with* calcifications in the group with the shortest duration (mean age 39 years) and 16 of 26 with calcifications in the group with the longer duration (mean age 40 years). The difference is still significant ($p < 0.02$). Of the 22 patients with calcifications 19 had calcifications on the films from the thigh or knee and 13 on the films from the foot.

Type of calcification present

Studying the films from the thigh and the knee it appeared that all patients had medial calcification and one showed in addition intimal calcification.

Relationship between maximal blood flow and calcification

The diabetics can be divided into two groups: those with a maximal blood flow below and those with a maximal blood flow above the regression line (Fig 1). In the low flow group there were 11 with calcifications in the thigh and knee regions and eight in the high flow group. This difference is not significant.

To get a more quantitative estimate of the relationship between the maximal blood flow and calcification the regression line was calculated for those with and those without calcification. In the group without calcification no association was found between maximal blood flow and duration of diabetes or between maximal blood flow and age. The same results were obtained when only those with a duration of diabetes of more than six years were included (see Figs 2 and 3 for explanation). However in the group with calcification there was a relationship between the maximal blood flow and duration of diabetes ($p < 0.002$) and between the maximal blood flow and the age of the patient ($p < 0.05$). The partial regression coefficients which relates maximal blood flow to duration of diabetes and the standard error is

Group without calcifications
-0.339 ± 0.353 ($p > 0.1$)
Group with calcifications
-1.347 ± 0.337 ($p < 0.002$)

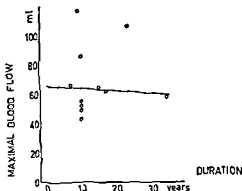


Fig 2 The relationship between maximal blood flow and duration of diabetes (group without calcification at the thigh and knee level)

The two slopes differ significantly ($p < 0.05$) The results are presented in Figs 2 and 3 For comparison all values from patients with a duration of diabetes of less than six years have been omitted from Fig 2

It was difficult to get a quantitative estimate of the arterial calcification However it can be said that the calcification was more extensive in patients with the longest duration of diabetes

Clinical vascular disease

Only three of the 75 patients had symptoms and signs of peripheral vascular disease in the legs Pulsation was absent in the dorsal pedal and posterior tibial arteries of these three patients and two of them had intermittent claudication The other patient had no complaints but walked very slowly The ages were 49 46 and 44 and the duration of diabetes 34 26 and 30 years These three patients had the lowest peak flow and the longest latency time

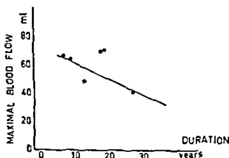


Fig 3 The relationship between maximal blood flow and duration of diabetes (group with calcification)

The latency time

The mean latency time i e time from release of the cuff pressure until the blood flow reached maximal value was 0.42 min ± 0.17 Latency time decreased with increasing duration of diabetes (the three patients with pulseless feet were excluded from this study) The equation of the regression line is

$$y \text{ (min)} = -0.005\ x + 0.48$$

and the slope of the line differs significantly from zero ($p < 0.01$) The standard deviation was ± 0.16 In the non-diabetic group the mean latency time was 0.47 min ± 0.18 This difference is not significant

Blood pressure

In the diabetic group the mean diastolic pressure during rest was 80 mm Hg $\pm$ 12 and the mean systolic pressure 130 mm Hg $\pm$ 18 ($n = 75$) After ischaemic work the diastolic pressure averaged 83 mm Hg $\pm$ 12 ($n = 65$) and the systolic pressure 137 mm Hg $\pm$ 19 ($n = 65$) The blood pressures increased with duration of diabetes Only the relationship between systolic pressure after ischaemic exercise and duration of diabetes was significant ($p < 0.05$) The equation of the regression line is

$$y \text{ (mm Hg)} = 0.527\ x_{(\text{ag })} + 0.450\ x_{(\text{duration})} + 112.5$$

The rise in systolic pressure after ischaemic exercise showed no significant association with duration of diabetes In the non-diabetic group the mean diastolic pressure at rest was 79 mm Hg $\pm$ 12 and after ischaemic exercise 84 mm Hg $\pm$ 12 The mean systolic pressure averaged 124 mm Hg $\pm$ 16 at rest and 131 mm Hg $\pm$ 18 after the ischaemic period Mean systolic blood pressures in diabetics and non-diabetics did not differ significantly

Tendon reflexes

In 21 patients or 28 % of the total material the achilles tendon reflex was completely absent Patellar reflexes were absent in ten patients (13) In the 38 patients with a duration of diabetes less than ten years tendon reflexes were absent in only one case Of the 37 cases with a duration of diabetes of more than ten years achilles tendon reflexes were absent in 20 and patellar tendon reflexes in nine A chi square test

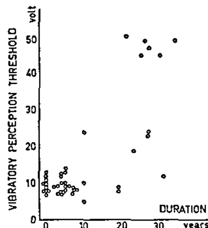


Fig 4 The relationship between the vibratory perception threshold and duration of diabetes

shows that this difference is significant ($p<0\ 001$ $p<0\ 002$)

Vibratory perception threshold

Fig 4 shows the relationship between vibratory perception threshold and duration of diabetes As can be seen from the figure the threshold remains fairly constant until the disease has lasted for about ten years After that time a very high proportion of the patients examined showed higher values For statistical analysis the results from the examinations of the threshold were converted according to the following equation (24)

$$z = 100 \log (\text{threshold} \ - 3)$$

Regression analysis was performed on the converted values with age and the duration of diabetes as the invariables The equation of the regression line is

$$y\ (\ )\ \ 34 + 1\ 372\ x_{(ag\ )} + 1\ 684\ x_{(duratio\ )}$$

The two partial regression coefficients differ significantly from zero ($p<0\ 001$) The total variance was 1263 and the residual variance 683

The mean threshold in the diabetic group was 18 and in the non-diabetic group 10 a significant difference ($p<0\ 001$ Mann Whitney U test)

Relationship between nervous system abnormalities and maximal blood flow

There was no obvious relationship in individual patients between maximal blood flow and vibratory perception threshold or between maximal blood flow and the presence or absence of reflexes To get a quantitative estimate multiple regression analysis was carried out When a correction was made for the age of the patient and the duration of diabetes there was a tendency for those with low flow to have a higher vibratory perception threshold than those without However the difference was not significant at the 5 % level although close to it ($t = 1\ 9605$ 5 % limit $= 1\ 9940$)

Other features

No association could be demonstrated between haemoglobin concentration ($n = 69$) and the duration of disease or between hæmoglobin concentration and maximal blood flow No association could be demonstrated between subcutaneous fat thickness ($n = 64$) and duration of disease or maximal blood flow Neither could any association be established between maximal working time and maximal blood flow The same results were obtained when the group with calcification was considered alone

DISCUSSION

The association between clinical vascular disease in the lower extremities and diabetes mellitus is well established In series of diabetics the frequency of vascular disease in the lower extremities is higher than in non-diabetics (1 4 21) In patients with vascular disease of the lower extremities the incidence of clinical diabetes mellitus is higher than in the population as a whole (15 18 23) It is also known that an abnormal glucose tolerance test is seen more frequently in patients with circulatory insufficiency of the lower extremities than in those without (25) However the observations mentioned above are mainly based on studies in older patients In younger diabetics clinical vascular disease is seen less frequently In the present study only three had symptoms and signs of vascular insufficiency in the legs

Earlier measurements of vascular function in the lower extremities of diabetics have concentrated on cutaneous blood flow Conflicting results have been obtained mainly because of the use of inappropriate methods and the fact that not all investigators have realised that a decreased vascular response may be due to auto-

nomic neuropathy as well to organic lesions in the vessels themselves The reports which deal with cutaneous blood flow will be reviewed else where (2)

There are few measurements of blood flow in the muscles of the lower extremities West et al (26) examined 60 diabetics and measured the vascular response in the calf after two minutes of ischaemic exercise by venous occlusion plethys mography No difference was found between nor mals and diabetics However no information is given about the duration of diabetes Munck et al (22) and Karlefors (16) measured the blood flow in the anterior tibial muscle by use of radio active xenon in 28 and 26 diabetics respectively and found identical values in the normal and diabetic group Munck et al (22) too failed to establish any difference when the diabetics were divided into those with nervous system abnormal ities (evaluated by examination of the tendon reflexes and measurements of the vibratory per ception threshold) and those without

The present results which were obtained in a larger series of patients demonstrate that the maximal xenon clearance decreases as duration of diabetes increases and this can with reason able confidence be taken as an expression of a decreased blood flow To some extent the xenon clearance does depend on haemoglobin concentra tion of the blood but no association could be demonstrated between haemoglobin concentration and duration of diabetes Xenon clearance also depends upon the fat content of the muscle studied No association could be demonstrated between subcutaneous fat thickness and duration of diabetes or maximal blood flow Opinions dif fer however as to the extent to which subcutane ous fat thickness can be used to measure mus cular fat content (9 10 13) Interstitial fat and vascular calcification are apparently not asso ciated (10) Lindbjerg et al (19) examined the fat content of the anterior tibial muscle in a small group of diabetics and found that the amount of fat did not differ from a group of non-diabetics

The decrease in maximal blood flow cannot be due to autonomic neuropathy as it is well known that reflexes are not involved in the dilatation of the vessels during ischaemia and exercise (5 6 11 14)

It is reasonable to conclud that the decreased blood flow found in diabetics is due to organic lesions of the vessels It was also shown that the decrease in maximal blood flow with increasing duration of diabetes was associated with the presence of arterial calcification

The increased frequency of calcification in the larger arteries has been demonstrated many times before (8 27) Only in the material presented by Ferrier (8) has an attempt been made to dis tinguish between medial and intimal calcifica tion The present study demonstrates as did Fer rier s that most of the calcification present in diabetics is medial

The decrease in latency time in diabetics which was first noted by Munck et al (22) is probably due to an increase in the rigidity of the vessels An increase in vessel rigidity in diabetics has also been suggested by the work of others using dif ferent methods (17 28)

Abnormalities of the nervous function could easily be demonstrated in the group presented here These observations are in accordance with those of other workers (7 12 24) and need not be discussed in detail except for mention of the demonstration by Gregersen (12) that the vibra tory perception threshold in diabetics is abnormal during the first year after diagnosis

In the present study it was not possible to demonstrate a significant relationship in the in dividual patient between maximal blood flow and vibratory perception threshold However flow was not measured in the nerves as this cannot be done at pres^nt and conditions might be dif ferent in the skin

It should be emphasized that the present study does not shed light on the problem as to whether clinical neuropathy meaning paresis pain and paraesthesia is due to ischaemia It is possible that clinical neuropathy as a disease only ap pears when a considerable ischaemic factor has been added to the altered metabolism of the nervous system which characterizes the diabetic state

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CONGENITAL PULMONARY STENOSIS AT THE AGE OF 76

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Abstract The record of a 76-year-old man with a congenital pulmonary stenosis is presented His cardiac function was studied by among other means heart catheterization which revealed a pressure difference at the pulmonary ostium of 27 mm Hg at rest and 40 mm Hg during light exercise

In discussing the prognosis of the patient with congenital pulmonary stenosis and when evaluating the indication for surgical correction of the abnormality the lack of appropriate control series has been apparent

To judge the severity of obstruction in a prognostic way long term observations of individual patients would be preferable Thus Engle et al (1) have described symptoms and signs and serial electrocardiograms of eleven patients for periods of 10–27 years without surgical intervention To our knowledge corresponding serial data of haemodynamic functions studied by heart catheterizations have not been reported

A more common approach has been to thoroughly examine the survivals in different age groups (1 2 5 6 7) which in the higher decades have been based upon casuistic data The purpose of this report is simply to add further information to the sparse data of these long standing lesions

CASE REPORT

A 76-year-old man was admitted to the hospital for investigation and treatment of what later appeared to be a non toxic thyroid adenoma On physical examination an arrythmia perpetua and a harsh systolic murmur were auscultated over the second left intercostal space The patient had a large thorax but no voussure or parasternal lift No signs of cyanosis or dyspnoea B P 140/80

A heart murmur was known to have existed since his youth and prevented him from performing his military service in 1910

His employment had been relatively heavy mostly installing machines Up to the last year he had been well and without symptoms The main complaints this last year had been of palpitations and dyspnoea on exertion He had received digitalis for about one year

The patient was referred to the Laboratory of Clinical Physiology for further investigation

The phonocardiogram showed a diamond shaped systolic murmur with punctum maximum over the second left intercostal space No diastolic murmur The first heart sound was normal The second heart sound was normal No splitting of the second heart sound could be detected an aortic component might however be hidden in the systolic murmur

On the X ray of the chest there was a marked enlargement of the pulmonary artery The peripheral vascular pattern was diminished The heart was enlarged 1400 ml corresponding to 705 ml/m BSA There was a moderate enlargement of the right ventricle The ECG at rest showed an atrial fibrillation an incomplete right bundle branch block and generalized ST depressions for which the digitalis medication might be responsible During the functional capacity test on a bicycle ergometer the patient worked 2 min at a load of 600 kpm/min with a pulse frequency of 1 4 beats/min. The test was interrupted because of a high breathing rate 36 c/min The patient experienced general fatigue and slight breathlessness

The total amount of haemoglobin (THb) was normal 950 g, and the heart volume was large when related to the THb

A heart catheterization was performed and the data (Table I) have been compared to normal values for the appropriate age (3 4)

At rest The cardiac output was ordinary in relation to the oxygen uptake the stroke volume was low

The systolic pressure of the right ventricle was elevated with a pressure difference of .7 mm Hg between the right ventricle and the pulmonary artery The end diastolic pressure of the right ventricle was above +2 S D from the mean value for the 8th decade The value of the pulmonary capillary venous pressure (PCV) fell between +1 and +2 S D from the appropriate mean value Ordinary pressures were measured in the brachial artery The calculated resistance of the pulmonary vascular bed was normal.

During exercise in supine position on a bicycle ergo-

Table I *Data obtained at rest and during exercise with right heart catheterization*

	Rest	200 kpm/min
Oxygen uptake ml STPD/min	318	725
Cardiac output l/min	5 2	6 4
Heart rate beats/min	72	93
Stroke volume ml	72	69
Pressures mm Hg		
Right atrium mean	13	
Right ventricle systolic	61	96
Right ventricle end-diastolic	14	19
Pulmonary artery systolic	34	56
Pulmonary artery mean	22	39
Pulmonary artery diastolic	15	29
Pulmonary capillary venous mean	14	21
Brachial artery systolic	138	205
Brachial artery mean	100	—
Brachial artery diastolic	69	80
Pulmonary resistance	1 6	0 6

meter the cardiac output increased less than expected from the oxygen uptake The stroke volume did not increase on transition from rest to exercise The systolic pressure of the right ventricle reached 96 mm Hg giving a pressure difference over the pulmonary valve of 40 mm Hg The end-diastolic pressure of the right ventricle was higher than normal at the same level of working intensity The PCV pressure was normal The pressures of the brachial artery were within normal limits, falling between +1 and +2 S D from the mean value The pulmonary resistance decreased

DISCUSSION

It seems reasonable to assume that the pulmonary stenosis of this patient is congenital Data of long standing valvular lesions are very rare and the surviving cases reported must be considered as positively selected

This patient had been without symptoms throughout his life up to the last year in spite of fairly heavy work up to the age of 65 The onset of the exertional dyspnoea might coincide with the appearance of atrial fibrillation Symptoms are however somewhat difficult to evaluate in patients with a congenital anomaly At the time of examination the stenosis was rather mild

The haemodynamic data differ from those in patients with severe pulmonary stenosis Mean pulmonary artery pressure and pulse pressure were not reduced and an ordinary cardiac output could be maintained at rest The oxygen saturation of arterial blood was normal

During work at a low load the small cardiac output was not appropriate to the oxygen uptake It is difficult to evaluate the relative importance of the valvular lesion and the atrial fibrillation for this hypokinetic circulation

As regards the aetiology of the atrial fibrillation it cannot be decisively referred to the vitium as cardiosclerosis cannot be excluded

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EFFECT OF A SINGLE DOSE OF NICOTINIC ACID ON PLASMA LIPIDS IN PATIENTS WITH HYPERLIPOPROTEINEMIA

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Abst act Eleven patients with uncomplicated ischemic cardiovascular diseases and moderate hyperlipoproteinemia were studied for 6 hours after the administration of one gram of placebo and two days later one gram of nicotinic acid

Plasma levels of free fatty acids were significantly depressed by nicotinic acid as compared to the control study during the first three hours after administration Then however there was a pronounced rebound of FFA The concentration of glycerol in plasma followed the same pattern as the free fatty acids

The values for cholesterol in plasma remained unchanged during the six hour period in both the placebo and the nicotinic acid study While the level of triglycerides as well was unchanged in the placebo study the concentration of triglycerides decreased significantly 4 and 6 hours after the administration of nicotinic acid

The concentration of blood glucose was not significantly different in the two groups during the study

The concentration of free nicotinic acid in plasma reached a peak value 30 to 60 minutes after nicotinic acid was taken by mouth At 5–6 hours the level had returned to pretreatment values The values for the plasma levels of free nicotinic acid and free fatty acids indicated that a concentration of nicotinic acid above 1 μg/ml plasma is needed to maintain low free fatty acid levels

The interrelation between free fatty acids triglycerides and cholesterol in plasma is discussed The results from these studies are compatible with the hypothesis that acute inhibition of FFA mobilization reduces the concentration of plasma triglycerides but not plasma cholesterol They do not explain the mechanism behind the cholesterol lowering effect of nicotinic acid seen after chronic administration

There is a strong association between high levels of lipids in plasma and ischemic cardiovascular diseases based on atherosclerosis such as myocardial infarction angina pectoris and intermittent claudication Retrospective studies have shown that groups of patients who have recovered from a myocardial infarction or who suffer from angina pectoris or intermittent claudication when compared to clinically healthy control subjects have hyperlipoproteinemia expressed as elevated levels of cholesterol and/or triglycerides in plasma (1 4 7) In Stockholm about 50 % of male patients with ischemic cardiovascular disease have an elevation of the concentration of either cholesterol or triglycerides in plasma above the upper normal limit (7 21) defined as the mean value +2 times the standard deviation for values obtained from clinically healthy men of comparable age (6) Prospective studies so far limited to plasma cholesterol have also indicated that the future incidence of cardiovascular ischemic disease is directly correlated to the concentration of cholesterol in plasma (23 25 26 28 29 30 33 36)

Treatment of the various kinds of hyperlipoproteinemia with the ultimate goal of reducing the incidence and clinical manifestations of atherosclerosis is gaining increasing interest There is no doubt that changes in our environmental conditions may have important effects on plasma lipid levels For instance the cholesterol level can be reduced by rather simple dietary means Such treatment has been reported to reduce the occurrence of coronary heart disease in healthy men (22) Treatment of survivors from myocardial infarction with a diet lowering plasma cholesterol levels has been shown to reduce the recurrence of new infarcts by about 50 % when the cholesterol level on the average was decreased by about 15 (31) However the more pronounced forms of hyperlipoproteinemia cannot be

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Table I *Age sex height weight plasma lipids blood sugar intravenous glucose tolerance (k value) and plasma FFA*

Case	Sex	Age (y)	Height (cm)	Weight (kg)	Cholesterol (mg/100 ml)	Phospholipids (mg/100 ml)	Triglycerides (mmol/l)	Fasting blood sugar (mg/100 ml)	k value[a] (/min)	FFA[b] (mEq/l) Placebo	Nic ac.
E. A	♀	58	171	70 2	428	431	3 27	61	1 35	0 40	0 63
K L.	♂	55	170	74 2	369	378	2 87	95	1 33	0 51	0 57
N O a	♂	72	170	63 2	391	454	2 37	100	1 33	0 37	0 40
S S	o	45	172	67 1	286	338	2 73	—	1 12	0 51	0 52
V C.	o	57	177	68 3	276	261	2 28	106	1 73	0 45	1 12
K S	♂	68	169	57 5	249	304	3 26	87	0 72	0 53	0 60
K H	♂	50	182	84	294	306	2 61	86	0 48	0 52	0 49
K. B	♀	47	162	59	271	300	1 85	95	0 47	0 98	0 47
S J	♂	44	161	61 8	343	314	4 14	99	0 65	0 49	0 31
K -A S	♂	54	171	84 2	451	496	9 97	105	1 08	0 66	0 37
N O b	o	57	—	86 0	191	235	1 59	82	1 01	0 83	0 63

[a] The *k* value was calculated from the apparent straight line in the semilogarithmic plot of blood glucose concentration against time after the intravenous injection of 50 g glucose Values above 1 10 normal and below 0 90 definitely pathological.
[b] The first value for FFA obtained in the placebo and in the nicotinic acid study is given

normalised by diet they need other treatment such as chemotherapy against their metabolic derangement Furthermore it has been argued that it is often difficult to change a patient s habits with regard to factors influencing plasma lipid levels such as diet exercise smoking and emotional stress even if the patient may be well motivated due to his disease (38) A chemotherapeutic compound acting without detriment to health and having a well established and sound mode of action would be a useful therapeutic agent in a study of the effect of a plasma lipid lowering program on mortality and morbidity of ischemic cardiovascular diseases

Nicotinic acid was shown in 1955 to lower plasma cholesterol levels by Altschul and co workers (3) and has since been used with increasing frequency in the treatment of hyperlipoproteinemia (2) We demonstrated in 1962 that nicotinic acid inhibits the mobilization of free fatty acids (FFA) from adipose tissue and postulated that this was a possible mechanism by which it reduced lipoprotein levels in plasma (9 19) An excessive rate of mobilization of FFA may be related to vascular disease in several ways e g by increasing the hepatic formation of triglyceride rich plasma lipoproteins by causing an increased uptake of fatty acids into the arterial wall and by possible influences on the process of thrombosis (13) We have demonstrated that acute exposure of men to emotional stress rapidly raises plasma FFA levels and within a few hours causes an increase in the content of triglycerides in plasma and that these reactions were inhibited by nicotinic acid (15) In addition to reduction of cholesterol concentration it is evident that nicotinic acid possesses the new pharmacologic principle for inhibition of FFA mobilization This effect is now well established (10 12) and might be of value in the treatment of hyperlipoproteinemia We decided to explore in detail different metabolic and clinical effects of nicotinic acid on patients with ischemic cardiovascular disease and with various types of hyperlipoproteinemia from the acute effect of a single dose to the effects of prolonged treatment

In this first communication patients have been observed for 6 hours after administration of a single dose of one gram of nicotinic acid or placebo

METHODS

All studies were done on hospitalized patients They all had ischemic cardiovascular disease either as a definite myocardial infarction (characteristic ECG and transaminase changes) at least 6 months before the study or as typical angina pectoris, with ECG changes during exercise su gesting myocardial ischemia or intermittent claudication with oscillometric recordings typical of obliterating arterial disease None had any complicating disease all were in good nutritional condition and the

Table II *Plasma levels of FFA (mEq/l) in the placebo and the nicotinic acid study*

Mean value ± standard error of the mean and range are given

Time h	0	½	1	1½	2	3	4	5	6
Placebo ($n=11$)									
M±S E M	59± 05	44± 03	42± 05	49± 06	60± 07	71± 06	76± 05	78± 05	78± 06
Range	40– 98	24– 60	24– 80	20– 81	17– 97	27–1 06	53–1 01	54–1 09	47–1 11
Nicotinic acid ($n=11$)									
M±S E M	53± 03	30± 02	22± 02	0± 0?	22± 02	31± 04	1 29± 16	1 71± 15	1 53± 17
Range	31–1 12	18– 41	14– 31	15– 32	12– 37	10– 55	52–2 08	1 04– 87	91–2 78

only symptoms present in some were ischemic pains on effort. No clinical diabetes with glucosuria and ketonuria was present although some patients had slightly elevated fasting blood glucose values and some had a lowered intravenous glucose tolerance in response to an intravenous glucose load (Table I) The patients had moderate hyperlipoproteinemia (hypercholesterolemia and/or hypertriglyceridemia) as set forth in Table I All patients had been followed by us at the outpatient department for at least several months, many for years During this time they had had repeated blood samples withdrawn for lipid analysis which showed that the lipid levels were fairly stable In no case did the lipid values at the time of the study show any deviation from the individual s usual level No patient was on any special diet at the time of the study and in the hospital they had the ordinary hospital d et Nor was any patient on drugs known to affect lipid metabolism or on antihypertens ve drugs They had not taken nicotinic acid earlier

The patients were carefully instructed about the purpose nature and poss ble side-effects of the study In the mornin, after fasting over night a teflon catheter was inserted into an antecubital vein A slow infusion of 0 9 saline kept the catheter patent and no heparin was used The patients who stayed in bed throughout the study were then gi en a light breakfast consisting of one cup of tea and two slices of bread without butter The main purpose of the l ght breakfast was to reduce possible gastro-intestinal discomfort caused by nicotinic acid They were then allowed only water to drink About 45-60 min after the breakfast the first blood sample was withdrawn and a one gram tablet given by mouth at the first study a placebo and at the second performed two days after the first, nicotinic acid as N cangin® (Draco Lund, Sweden) Blood samples about 10 ml each time were then drawn into heparinized syringes and in most instances without stasis Blood was immediately precipitated for glucose determination and the rest stored in ice water brought to the laboratory and processed within a few hours

Plasma FFA was determined according to Dole (4) as modified by Trout et al (37) cholesterol accord ng to Sperry Webb (35) and tr glycerides by the method of Carlson and Wadstrom (0) as modified by Carlson (8) Blood glucose (3) and plasma glycerol (39) were determ ned enzymat cally Plasma prote ns were estimated in tr pl cate by the biuret method All lipid values in plasma were corrected for the minor changes in protein concentration that occurred The concentration of *free* nicotinic acid in plasma was also determined in some studies (11) Statistical calculations were made as recommended by Snedecor (34)

RESULTS

Plasma FFA

The mean values are given in Table II and shown in Fig 1 When placebo was given the concentration of FFA fell at 30 and 60 min presumably as a result of the light breakfast From one hour onwards the FFA level rose slowly and remained fairly constant during the last two hours In the nicotinic acid study the initial values were not

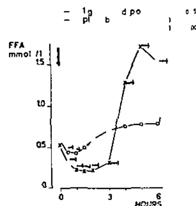


Fig 1 Effect of n cotinic acid on free fatty acids (FFA) of blood plasma in 11 patients w th ischemic-cardiovascular disease The subjects were first given placebo (O — O) and two days later 1 g of n cotinic acid (× — ×) by mouth as indicated in the figure The *statistical calculations were made on the indi idual changes* from the FFA concentration at zero time The P values ind cate the stat stical signif cance for the changes from zero time

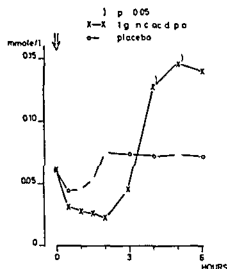


Fig 2 Effect of nicotinic acid on plasma glycerol in 8 patients For details see text to Fig 1

significantly different from those in the placebo study Nicotinic acid rapidly depressed the FFA level and already after 30 min the level was significantly below the placebo value The FFA level remained significantly depressed for 3 hours and then increased rapidly into an "overshoot" and was significantly elevated above the initial value and also above those for the controls

Plasma glycerol

The concentration of glycerol is shown in Fig 2 Glycerol followed the same pattern as FFA after both placebo and nicotinic acid but showed greater variability and was studied in fewer cases The changes in glycerol levels from the initial values were only statistically significant during the overshoot period at 4 and 5 hours However when comparing the individual glycerol levels after nicotinic acid and after placebo it was found that nicotinic acid caused a significant decrease in glycerol The mean individual differences between the two studies were after 1 1/2 2 and 3 hours 0.026 ± 0.010 ($P < 0.05$) 0.054 ± 0.012 ($P < 0.01$) and 0.025 ± 0.010 ($P < 0.05$) mmol/l respectively

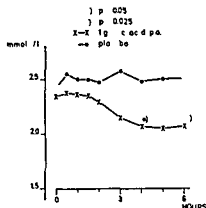


Fig 4 Effect of nicotinic acid on plasma triglycerides in 11 patients For details see text to Fig 1

Plasma cholesterol

The mean initial concentration of cholesterol was 313 ± 24 mg/100 ml in the placebo study and 302 ± 25 mg/100 ml in the nicotinic acid study *During both studies the cholesterol level remained* unchanged during the 6 hour period as indicated in Fig 3 The average change from 0 to 6 hours in concentration of cholesterol and its standard error of mean was 9 ± 7 mg/100 ml after placebo and 0 ± 6 mg/100 ml after nicotinic acid

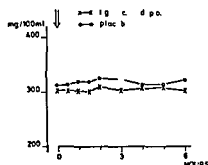


Fig 3 Effect of nicotinic acid on plasma cholesterol in 11 patients For details see text to Fig 1

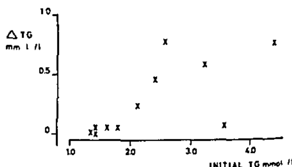


Fig 5 Effect of nicotinic acid on plasma triglycerides in 11 patients The figure shows the relation between the changes in plasma triglyceride concentration 6 hours after 1 g of nicotinic acid by mouth and the triglyceride concentration at zero time

Table III *Plasma levels of FFA (mEq/l) and nicotinic acid (μg/ml) after 1 g Nicangin® by mouth at 0 hours*

Case	Time h	0	½	1	1½	2	3	4	5	6
E. A.	FFA	0 65	0 34	0 27	0.23	0 37	0 54	1 82	1 80	1 49
	Nic	0 3	15 9	28 7	26 0	13 0	1 9	0 2	0	0
K. L.	FFA	0 57	0 28	0 20	0 2..	0 25	0 26	1 93	2 87	2 78
	Nic	0 1	17.5	29 3	26 0	18 4	4 1	1 0	0 5	0 3
N O b	FFA	0 63	0 38	0 23	0 17	0 15	0 10	1 11	1 47	1 31
	Nic	0	30 8	34 0	23 0	15 7	3 2	0 9	0 5	0 2
S S	FFA	0 52	0.27	0 21	0 17	0 15	0 55	2 08	2 03	1 20
	Nic	0	38 5	26 7	16 3	10 0	1 4	0 6	0 2	0 2
K. S	FFA	0 60	0 39	0 31	0 32	0 34	0 32	0 66	2 06	2 14
	Nic	0 4	34 0	28 4	32.1	22.6	9 7	2 6	0 9	0 3
K. H	FFA	0 49	0 30	0 18	0 19	0 18	0 23	1 07	1 04	0 91
	N c.	0 4	9 4	20 9	19 5	12 9	4 9	1 2	0 7	0 4
K. B	FFA	0 47	0 27	0 21	0 15	0 18	0 27	1 44	1 47	1.37
	Nic	0	32 2	24 1	19 0	11.2	1 4	0 1	0 2	0 1
S J	FFA	0 31	0 20	0 14	0 15	0 21	0 20	0 96	1 13	1 05
	Nic	0 2	32 9	33 0	31 7	15 4	3 3	1 0	0 3	0
K. A S	FFA	0 57	0 25	0 21	0 18	0 19	0 28	0 52	1 71	1 99
	Nic	0	23 6	23 0	20 0	14 0	4 3	1..	0 1	0
Mean	*FFA*	*0 53*	*0.28*	*0 22*	*0 20*	*0 22*	*0 31*	*1 29*	*1 73*	*1 58*
	Nic	0 1	26 1	27 6	23 7	14 8	3 8	1 0	0 4	0 2

Plasma triglycerides

The triglyceride level was 2 44 ± 0 30 and 2 36 ± 0 30 mmol/l at the beginning of the placebo and the nicotinic acid study respectively After administration of placebo the concentration of triglycerides did not change as shown in Fig 4 After nicotinic acid however there was a fall beginning after 2 hours from around 2 4 to 2 1 mmol/l which was significant at 4 and 6 hours The average change in the concentration of triglycerides and its standard error of mean after 6 hours was +0 06 ± 0 06 mmol/l ($P > 0$ 05) and −0 28 ± 0 09 mmol/l ($P < 0$ 025) in the placebo and the nicotinic acid group respectively The same statistical significance was obtained when the calculations were performed on logarithmic values (6)

The effect on the plasma triglycerides was generally related to the triglyceride concentration Fig 5 shows that the patients with triglyceride levels below 2 mmol/l had fairly small decreases 6 hours after administration of nicotinic acid Patients with higher triglyceride concentration generally exhibited more pronounced effects

Blood glucose

The behaviour of blood glucose was identical in the two groups The initial values were 99 ± 5 and 96 ± 4 mg/100 ml (mean ± S.E.M.) in the placebo and the nicotinic acid study At 2 4 and 6 hours the corresponding mean values in these two studies were 90 and 86 78 and 80 73 and 75

Free nicotinic acid in plasma

In nine patients the concentration of free nicotinic acid was determined in plasma These values and the corresponding FFA values are listed in Table III Free nicotinic acid reached a peak value of around 30 μg/ml already after 30 min in four of the patients and at one hour in the rest After one hour the concentration started to fall first slowly and then more rapidly At three and four hours the plasma level of nicotinic acid had fallen to around 4 and 1 μg/ml respectively Inspection of Table III gives some information about the relationship between plasma levels of free nicotinic acid and the effect on FFA mobilization Thirty min after nicotinic acid had been taken the FFA level had decreased in all cases and at that time the lowest concentration of free nicotinic acid was 9 4 μg/ml If we consider an increase in FFA concentration of 0 10 mEq/l significant we may say that the FFA level was still depressed at an average nicotinic acid concentration of 8 8 μg/ml (range 1 4–26 0) but had started to rise on the average at a nicotinic acid concentration of 2 7 μg/ml (0 1–13 0)

Table IV *Symptoms after administration of 1 g nicotinic acid by mouth at 0 hours*

Case	Time h	½	1	1½	2	3	4	5	6
E. A	Flush	+ +	+ + +	+ +	+	–	–	–	–
	Other					C			
K L.	Flush	+ + +	+ + +	+ +	–	–	–	–	–
	Other					C			
N O a	Flush	+ + +	+ + +	+ +	+	–	–	–	–
	Other	T	T	T	T	T C	T	T	
S S	Flush	+ + +	+ +	+	–	–	–	–	–
	Other					C			
V C	Flush	+ + +	+ +	–	–	–	–	–	–
	Other				C				
K S	Flush	+ +	+	–	–	–	–	–	–
	Other				C				
K H	Flush	+ + +	+ + +	+ +	+	–	–	–	–
	Other						C		
K B	Flush	+ + +	+ +	–	–	–	–	–	–
	Other				C				
S J	Flush	+	–	–	–	–	–	–	–
	Other								
K A S	Flush	+ + +	+ + +	+ +	–	–	–	–	–
	Other				C	V			
N O b	Flush	+ + +	+	+	–	–	–	–	–
	Other					C			

C = Chilly T = Tinnitus V = Vomiting – = No visible flush + = Slight flush in the face + + = Marked flush in the face neck and upper part of the body + + + = Marked flush in the face and the whole body

Symptoms

Nicotinic acid causes a pronounced flush when given for the first time to a human being This effect is significantly reduced or even disappears during long term treatment One gram of nicotinic acid as a single dose is usually the minimal dosage used during treatment of hyperlipoproteinemia However as a first dose one gram is an unusually high dose The symptoms produced by this high dose are therefore of clinical interest and are recorded in Table IV All patients flushed One had flush only in the face all the others over the entire body The maximal flush appeared already after 30 min in all but one of the patients The flush had disappeared completely within 3 hours With the exception of the patient having flush only in the face all cases felt cold when the flush had disappeared

DISCUSSION

Our main interest in this study concerns the relationship between the levels of plasma FFA and the concentration of cholesterol and triglycerides in plasma One gram of nicotinic acid in comparison to administration of placebo lowered the FFA level significantly for three hours and reduced the concentration of triglycerides at 4 and 6 hours The cholesterol level however remained unchanged during the entire study in both groups This suggests that only one of the three main plasma lipoprotein classes had been affected by nicotinic acid namely the very low density class The very low density lipoproteins have triglycerides as their main constituent and these lipoproteins are believed to transport fatty acids as triglyceride fatty acids *from the liver* to peripheral tissues In the fasting state FFA

from adipose tissue are the main precursor to the fatty acids of the very low density lipoprotein triglycerides It is thus conceivable that at least in the fasting state lowering of FFA mobilization from adipose tissue should decrease the liver uptake of FFA and consequently the hepatic formation of the triglyceride rich very low density lipoproteins Our results strongly support this hypothesis In the fasting rat we have previously demonstrated similar effects Subcutaneous administration of nicotinic acid to rats lowered FFA levels for about four hours and decreased the plasma triglyceride concentration within 2 hours by more than 50 per cent (14 18) In these studies analysis of liver lipids revealed that also the hepatic triglyceride content had been reduced by more than 50 in analogy with the hypothesis set forth above Experiments with stimulation of FFA mobilization also strongly support the hypothesis relating the rate of mobilization of FFA to plasma triglyceride metabolism Increasing FFA mobilization by noradrenaline rapidly increases the amount of triglycerides in the liver (16 17 27) and raises their concentration in plasma (17) This increase in plasma triglyceride concentration was demonstrated to be due to an increase in the content of very low density lipoproteins (17)

That the relationship between FFA mobilization and plasma lipoprotein levels has a not only academic interest but may be related to every day situations was borne out by our studies with a standardized emotional stress procedure which may well correspond to situations in everyday life It was found that exposure of men for two hours to such a procedure rapidly increased FFA levels and raised triglyceride but not cholesterol concentration in plasma (15) In conformity with this study administration of nicotinic acid largely prevented the stress induced FFA increase and lowered plasma triglycerides but not cholesterol (15)

This study does not answer the question how chronic treatment with nicotinic acid lowers the concentration of cholesterol in plasma which was the effect first found with nicotinic acid on plasma lipids 12 years ago (3) Our data demonstrate that nicotinic acid first lowers FFA levels in plasma and then triglycerides The effect on plasma cholesterol must be a later event Whether or not the effect on cholesterol may be related to the inhibition of FFA mobilization will be studied later on

When the concentration of nicotinic acid in plasma had fallen to around 3 μg/ml the FFA levels started to rise This may appear somewhat surprising compared to studies with adipose tissue incubated in vitro In studies with rat (5 9) and human (10 12) adipose tissue nicotinic acid had a maximal inhibitory action on FFA mobilization at a concentration of around 0 1 μg/ml This concentration is apparently about 10 times less than the plasma concentration needed in vivo to obtain maximal suppression of FFA mobilization However further in vitro studies in which whole serum was used as incubation medium instead of the usual albumin Krebs Ringer buffer solution showed that in the medium with serum in fact much higher concentrations of nicotinic acid were needed than in albumin media to inhibit lipolysis (12) We have no explanation of the discrepancy between these two media but it was shown that binding of nicotinic acid to serum proteins could not be a factor of importance in this connection (12)

The rather pronounced FFA rebound occurring at 4 to 6 hours after 1 gram of nicotinic acid had been given by mouth deserves attention and clinical consideration An overshoot in the FFA levels after nicotinic acid was evident in the first three published cases in which smaller doses had been given (19) The cause of this rebound is not known It may be elicited locally in adipose tissue by operation of local feedback mechanisms or may be due to some systemic reaction to nicotinic acid The overshoot may partly explain why some patients need so large and frequent doses of nicotinic acid in order to keep lipoprotein levels low The overshoot is very likely not a desirable therapeutic effect and will be studied in more detail

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C REACTIVE PROTEIN IN A RANDOM SAMPLE OF SWEDISH MEN AGED FIFTY

Distribution and Relation to Clinical Manifestations[1]

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Abstract The CRP concentration has been determined by means of immunodiffusion techniques in blood sera from a random sample of 876 men aged fifty The prevalence rates of sera with CRP concentration equal to or higher than 1–4 2 μg/ml increased with increased tobacco consumption, severity of symptoms of respiratory disease and elevated sedimentation rate In contrast to this the prevalence rate of sera containing trace amounts of CRP decreased as these variables increased It is suggested that the presence of trace amounts of CRP in several consecutive samples from an individual is of minor clinical significance but may rather indicate changes associated with aging.

The occurrence of C reactive protein (CRP) in various clinical disorders has been analyzed by many authors (for reviews see 7 14) In most of these studies the capillary tube precipitation test (1) has been used The extensive clinical studies of Hedlund (5) were mostly performed with the non specific capsular swelling reaction elaborated by Löfstrom (8) Neither of these two techniques however gives more than a semiquantitative estimation of the CRP content of the sera The greater sensitivity quantitative accuracy and specificity of immunodiffusion techniques for CRP determination has been shown by Nilsson (10)

The aim of the present study was to examine the distribution of CRP and to relate the CRP concentration of blood serum as determined by immunodiffusion techniques to some other laboratory and clinical variables in a random sample of men aged fifty Especially the clinical significance of low CRP concentrations as demonstrated by the immunodiffusion methods employed was considered in relation to these variables

[1] Part of these results were presented at the XXX Scandinavian Congress of Internal Medicine Abo 1966

MATERIAL AND METHODS

During 1963 a combined health examination and population study of 973 randomly selected men was performed at the Sahlgren Hospital in Goteborg The investigated series was selected from the register of the Revenue Office and consisted of the entire male population born in 1913 on dates which are multiples of three and who were living in Goteborg at the time of the study A 4 hour examination of the individual s overall state of health was performed exploiting the personnel and technical resources of a large city hospital Eight hundred and fifty five participants (88%) were examined in the hospital The entire series including the individuals who were not examined in hospital has been analyzed by Tibblin (15)

The classification of individuals with regard to signs of chronic bronchitis as well as the presence or absence of other respiratory symptoms was that described by Tibblin and Wilhelmsen (16) In their study a translation of the questionnaire on respiratory symptoms which has been approved by the British Medical Research Council s Committee on the Aetiology of Chronic Bronchitis (3) was used The series was thus divided into the following three groups

I Subjects without respiratory symptoms
II Subjects with signs of respiratory disease other than chronic bronchitis
III Subjects with signs of chronic bronchitis (cough and sputum for at least three months during the year)

The subjects were also divided into groups with regard to daily consumption of tobacco as described by Wilhelmsen and Tibblin (18)

Blood sedimentation rate in 1 h was determined by Westergren s method The test was done within 1 h of the blood being drawn

Paper electrophoresis of serum samples was performed in tris buffer pH 8 9 according to Aronsson and Grön

wall (7) The total serum protein was determined by the Biuret method These investigations were performed on the day that the subjects were examined. All analyses of serum proteins were made by the same person.

At the time when the CRP analyses were undertaken 8_6 of the original deep-frozen sera were available for study This series comprised essentially group A of a previous study (10) in which the CRP determinations were performed by means of the capillary tube precipitation test and the double diffusion-in gel technique The CRP concentration was determined by the single radial diffusion (halo) technique as described by Mancini et al. (9) slightly modified (10) The halo technique allowed accurate quantification of as low concentrations as 1 µg CRP/ml serum. With the comparative double diffusion in-gel techniqu concentrations as low as approximately 0.5 µg ml could be demonstrated. The sera were grouped according to CRP content in the following way

Negative
Sera without demonstrable CRP as determined by the comparative double diffusion in gel technique

Trace amounts
Sera giving a positive reaction in the double diffusion-in-gel technique but in which the CRP concentration was too low to be estimated by the halo technique (< 1 µg/ml)

More than 1 µg CRP/ml
Sera giving a positive reaction in the double diffusion in-gel technique and also quantifiable with the halo technique. These sera were arbitrarily divided into three groups (1–4.2, 4.3–_5.4 and _5.5–89 µg CRP/ml) before analysis of data. In order to examine the frequency distribution of sera with different CRP concentrations the group containing 4 3–25 4 µg ml was further divided into four subgroups.

Computer analysis (SAAB D _1) was performed to facilitate the comparison of CRP concentration with the other variables under investigation.

RESULTS

A frequency distribution of the CRP concentrations in the 826 subjects included in the present report is given in Fig 1 It can be seen that in 134 sera (16 2 %) no CRP could be shown whereas in 428 (51 8 %) trace reactions were obtained One hundred and forty two sera (17.2 %) had a CRP concentration between 1 and 4 2 µg/ml and in 122 sera (14 8 %) more than 4.2 µg CRP/ml was demonstrated Thus the presence of CRP was demonstrated in a total of 83 8 % of the men included in this series

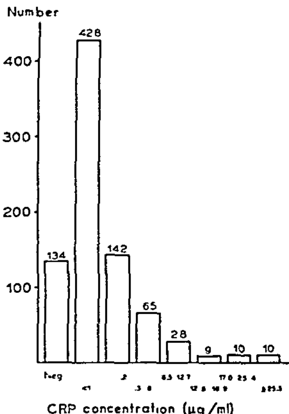


Fig 1 Frequency distribution for 8?6 randomly selected subjects aged fifty with different CRP concentrations as determined by immunodiffusion techniques

Table I *Prevalence rate of CRP in 8?6 randomly selected subjects aged fifty with different smoking habits*

CRP concentration (µg ml)	Non-smokers		Ex-smokers		Pipe and cigar smokers		1–14 cig./day		15–24 cig /day		≥ 25 cig day		Total
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	
≥25.5	2	1 0	1	0 6	0	0	1	0 4	4	3 0	2	6 7	10
4.3–25 4	14	7 1	15	9 1	13	17 8	32	14 2	29	21 5	9	30 0	112
1–4 2	32	16.2	25	15.2	11	15 1	32	14.2	37	27 4	5	16 7	142
<1	108	54 5	93	56 4	36	49.3	1?6	55 8	52	39 3	13	43 3	4 [illegible]
Negative	4?	21.2	31	18 8	13	17 8	35	15.5	12	8 9	1	3 3	134
Total	198	100 0	165	100 1	73	100 0	226	100 1	134	100 1	30	100 0	8 6

Table II *Prevalence rate of CRP in 826 randomly selected subjects aged fifty with or without different respiratory symptoms*

CRP con centration (μg ml)	I Normal		II Resp symptoms		III Chronic bronchitis		Total
	n		*n*	%	*n*		
≥25 5	5	1 1	4	1 4	1	1 6	10
4 3–25 4	54	11 4	45	15 6	13	20 6	112
1–4.2	67	14 1	6	21 5	13	20 6	142
<1	257	54 1	143	49 7	28	44 4	428
Negative	92	19 4	34	11 8	8	12 7	134
Total	475	100 1	288	100 0	63	99 9	826

Table IV *Mean values (g/100 ml) of total serum protein and electrophoretic fractions in subjects with different CRP concentrations*

CRP con centration (μg/ml)	Total protein	Albumin	α_1	α	β	γ
>25 5	7 10	3 81	0 40	0 59	1 09	1 33
12 8–25 4	7 25	4 18	0 40	0 64	1 11	1 02
8 5–12 7	7 05	4 27	0 40	0 56	0 94	0 96
4 3–8 4	7 15	4 14	0 38	0 58	1 04	1 10
1–4 2	7 22	4 21	0 36	0 54	1 03	1 17
<1	7 10	4 18	0 36	0 52	1 02	1 12
Negative	7 12	4 16	0 34	0 51	1 00	1 18

Table I shows the prevalence rates of sera with varying CRP concentrations in subjects with different smoking habits There was an increase in prevalence rate of sera with CRP concentrations exceeding 4 2 μg/ml as the tobacco consumption rose A decrease in prevalence rate of sera with trace amounts and sera without demonstrable CRP was noted with increased tobacco consumption The prevalence rates of sera with different CRP concentrations among the pipe and cigar smokers were about the same as in the group smoking 1–14 cigarettes per day

In Table II are seen the prevalence rates of sera with varying CRP concentrations in subjects with or without respiratory symptoms It is seen that the prevalence rate of subjects with a CRP concentration exceeding 4 2 μg/ml increased with increasing severity of respiratory symptoms The prevalence rate of subjects with a CRP concentration between 1 and 4 2 μg/ml was about the same (21%) in both groups with respiratory symptoms and about $^2/_3$ of this rate in the group lacking respiratory symptoms Subjects showing negative or trace CRP reactions on the other hand had a decreased prevalence rate with more accentuated symptoms of bronchitis

In Table III the prevalence rates of sera with different CRP concentrations in subjects grouped according to sedimentation rate are recorded The prevalence rates of sera containing CRP concentrations exceeding 1 μg/ml increased with increasing sedimentation rate On the contrary there was a decreasing prevalence rate of sera showing only trace amounts or with no demonstrable CRP with higher sedimentation rate

Finally in Table IV are recorded the mean values of total serum protein and electrophoretic fractions in subjects with different CRP concentrations it may be noted that there was a slight increase of α_1 and α globulins with increasing CRP concentration

Table III *Prevalence rate of CRP in 821 randomly selected subjects aged fifty with different sedimentation rates*

CRP con centration (μg ml)	Sedimentation rate mm/h <10		≥10 ≤14		≥15		Total
	n		*n*		*n*		
≥25 5	3	0 5	1	0 9	6	6 6	10
4 3 25 4	62	10 1	22	18 8	27	29 7	111
1–4..	90	14 7	25	21 4	25	27 5	140
<1	338	55 1	61	52 1	28	30 8	4"7
Negative	120	19 6	8	6 8	5	5 5	133
Total	613	100 0	117	100 0	91	100 1	8 1

Value of sedimentation rate missing in f' e subjects

DISCUSSION

The demonstration of CRP in various biological fluids has be n shown to be a sensitive indication of an active inflammatory or necrotic process in the organism Reviews on the occurrence of CRP in various diseases have been published by e g Hedlund (5) Kettel et al (7) and Schwarz (14) Several reports have been devoted to the relation between the presence of CRP and other laboratory findings also indicating inflammation such as elevated sedimentation rate pathological changes of various electrophoretic serum fractions glyco-

proteins AST-O etc (for review see 14) Only in some of these studies has quantification of CRP been performed In most of the studies groups selected with regard to types of disease have been analyzed whereas the results of the present study are based on a representative series from a population of men aged 50 Since the immunological techniques used for demonstration and quantification of CRP in the present investigation have been shown to be more sensitive than the capillary tube precipitation test and probably do not give any false positive reactions (10) it was considered worthwhile to investigate the relationship between the CRP concentration and certain clinical and laboratory parameters Especially it seemed of interest to examine the possible clinical significance of the presence of low concentrations of CRP in blood sera since these are not detected by the routine capillary tube precipitation test Although the principal aim of the clinical part of the study (15) was to elucidate early signs of latent or chronic diseases it was felt that this randomly selected material would be suited to this purpose as well

Due to difficulties in finding an acceptable statistical model which was also surveyable statistical interpretation of the findings cannot be presented The analyses revealed the presence of CRP in the blood sera of 83 8% of the subjects investigated The majority of the participants were in active work and showed on clinical examination a rather low incidence of apparent disease (15 table 26) With this in mind and the fact that the series was obtained from a randomly selected group this prevalence rate can be considered high That seasonal variations of the incidence of e g infectious diseases should be responsible for the high prevalence rate of CRP containing sera is unlikely since the serum samples were collected throughout the year except for the last days of June and the whole of July When the sera were tested with the capillary tube precipitation test CRP was demonstrated in 25% The demonstration of CRP at a prevalence rate as high as 84% is probably due to the greater sensitivity of the employed technique (10)

The prevalence rate of sera with CRP concentrations exceeding 4 2 μg/ml increased with increased tobacco consumption (Table I) whereas the prevalence rates of sera with trace amounts and with no demonstrable CRP decreased with increasing tobacco consumption These results are in accord with those reported by Heiskell et al (6) These authors demonstrated a certain relationship in males between smoking and the presence of CRP When different age groups were analyzed a closer relationship was indicated between smoking and CRP in both males and females However the present study indicates the usefulness of a quantitative determination of CRP at least when sufficiently sensitive techniques are used As regards the cause of this relationship an excessive morbidity "due to a wide variety of etiologically unrelated diseases" in smokers has been considered (6) Since the occurrence of CRP seems to be intimately related to inflammatory conditions it seems most probable that the chronic bronchitis accompanying smoking may be of importance in this connection This is also in accord with the figures presented in Table II in which is seen the higher prevalence rate of sera with CRP concentrations exceeding 1 μg/ml in subjects with respiratory symptoms compared to those without such symptoms These results are in accord with those reported by Colao (4) in patients with chronic bronchitis In Table II are also seen the decreasing prevalence rates of traces and negative reactions with increasing severity of respiratory symptoms

The relationship between elevated sedimentation rate and the presence of CRP in serum has been the subject of many reports Most authors however seem to agree that CRP is a sensitive indicator which more rapidly reflects changes of an inflammatory or necrotic process than the sedimentation rate (7 12 13 17) The present series revealed an increasing prevalence rate of sera with CRP concentrations exceeding 1 μg/ml whereas there was a decreasing prevalence rate of sera containing trace amounts and negatively reacting sera with increasing sedimentation rate

The trends indicated in Table IV are in accord with the earlier reported relationship between the presence of CRP and elevation of α_1 and α_2-globulins (e g 7 17)

The prevalence rates of sera containing trace amounts of CRP never increased with any of the variables tested but tended rather to decrease as did those of sera without demonstrable CRP Judging from these results the presence of trace amounts may not be indicative of an active pro-

cess It should be remembered that the present investigation is a population study in which each individual is represented by one serum sample and that conclusions of this kind might not be applicable to the course of a disease in an individual subject It is possible that in individuals showing trace amounts of CRP follow up testing would reveal an incipient or declining disease Anderson and McCarty (1) and Wood and McCarty (19) are of the opinion that the use fulness of the CRP test for the evaluation of rheumatic activity is greatest in the lower CRP concentration range The difference in sensitivity between the capillary tube precipitation test and the immunodiffusion techniques should be considered Another study performed by one of the present authors in a series of blood donors indicated that the prevalence rates of sera with low concentrations of CRP (<4 2 μg/ml) increase with increasing age (11) It might be that the presence of these low concentrations of CRP in several consecutive samples indicate a CRP level which could be associated with changes in the aging organism

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IMMUNOGLOBULINS IN THE COURSE OF VIRAL HEPATITIS AND IN CHOLESTATIC AND OBSTRUCTIVE JAUNDICE

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Abstract Immunoglobulins G A and M were determined during the course of disease in 19 cases of sporadic infectious hepatitis eight cases of epidemic infectious hepatitis 11 cases of serum hepatitis five cases of cholestatic jaundice and 15 cases of obstructive jaundice A marked increase was found initially in both infectious hepatitis groups (367% ±160 and 376 ±145 respectively) which fell precipitously during the first weeks of illness Significantly lower γM levels were found in the serum hepatitis group (210% ±87) and five of the cases showed normal immunoglobulin levels

γG levels rose to a lesser extent (158 ±54 and 142% ±36) in the infectious hepatitis patients but tended to remain elevated longer There was no significant difference between infectious hepatitis and serum hepatitis (131 ±37) Small but significant increases in γA levels were observed in infectious hepatitis

Slight or no changes occurred in γD levels in three cases of infectious hepatitis and two cases of serum hepatitis No alterations of immunoglobulin levels were observed in connection with the onset of cholestatic or obstructive jaundice indicating that liver damage per se does not give rise to the changes observed in viral hepatitis

A close correlation was observed in this material of previously healthy individuals between thymol turbidity and γM ($r = 0.76$)

It is well established that infectious hepatitis (IH) in most instances is accompanied by a polyclonal type of hypergammaglobulinemia (9) lasting from one to several months (5) Heremans (12) was first to publish levels of the three classes of immunoglobulins G A and M in a few cases of IH Increased γM levels was the most constant finding Recently a number of authors have reported results that on the whole confirmed those of Heremans (2 8 15 16 17 21) Except for four cases studied by Lee (15) with weekly examination the patients were examined only once or a few times during the course of illness No corresponding studies of serum hepatitis (SH) cholestatic jaundice or obstructive jaundice are known to the author

It is the aim of this paper to study the immunoglobulins G A and M in patients with IH as well as SH during the acute illness and recovery and also in cases of cholestatic and acute obstructive jaundice The diagnostic and prognostic value of these determinations will be investigated Furthermore the γD levels will be reported in selected cases of IH and SH Finally the correlation between immunoglobulin levels and the thymol turbidity test is studied

Abbreviations

AHG = antihemophilic globulin GT = γ glutamyltranspeptidase SGPT = serum glutamic puruvic transaminase

METHODS

Immunoglobulin levels were determined by immunodiffusion in Oudin tubes with specific antisera as previously described (4 24) All results were expressed in per cent of a pooled standard serum The sera were frozen within eight hours and kept at −20 C until used A few sera were analyzed fresh and again after one year at −20 C The results agreed within 10% The following tests were performed on fresh specimens in the routine laboratory of clinical chemistry (normal values in parentheses) bilirubin (0 2–1 1 mg/100 ml) thymol turbidity test (absorbency 0 0–0 11) SGPT (7–30 Karmen units) alkaline phosphatases (2–8 Buch and Buch units) γ-glutamyltranspeptidase (25–100 units)

MATERIAL

In 19 cases (ten men and nine women) a diagnosis of sporadic IH was based on a typical clinical picture with prodromal symptoms fever jaundice and recovery The mean age was 25 years Twelve of the patients had recently spent some time as tourists in Mediterranean

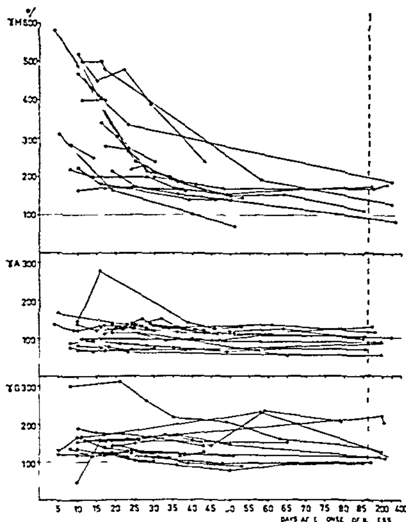


Fig 1 γG γA and γM levels in 19 cases of sporadic infectious hepatitis. Values in per cent of a standard pool serum containing approximately 1000 mg% γG 200 mg% γA and 100 mg% γM

countries This is considered to constitute a definite risk for acquiring IH for Scandinavians None of the 19 individuals in this group had received prophylactic gammaglobulin injections Typical laboratory data included serum bilirubin concentrations peaking between 2 7 and 19 5 mg 100 ml SGPT ranging between 16 and 25 0 Karmen units and initial thymol turbidity between 0 17 and 0 94 However two cases with otherwise typical data had normal thymol turbidity

Another group of IH patients belonged to a family consisting of two sisters, 38 and 3_ years old, and six of their ten children aged 7 to 14 This epidemic was thought to have originated in a 13 year-old daughter who had been ill with an unicteric febrile illness with abdominal discomfort three weeks prior to the outbreak in the eight cases. On examination no abnormality was found except for a thymol turbidity of 0 1_ Among the eight cases two children had unicteric but otherwise typical disease Peak bilirubin levels ranged between 0 5 and 6.9 mg/100 ml, thymol turbidity between 0.24 and 0 70 and SGPT levels between 135 and 1944 All cases had a normal recovery

In 11 cases a diagnosis of SH was made This group consisted of eight men and three women, the mean age was 31 years (range 15 to 67) Five of the patients were drug addicts who had injected themselves under poor hygienic conditions 65 to 1_0 days prior to the onset of jaundice One patient had undergone tattooing in a prison 1 0 days before falling ill One woman had been in contact with hepatitis-contaminated glassware 60 days before the illness when she had an ulcer on a finger Finally four patients had received whole blood or AHG 5_ to 130 days before onset. The onset in many of these cases was more insidious and the course in some cases more protracted The peak bilirubin levels were between 1 5 and 14 mg 100 ml SGPT levels between 34_ and 1950 Whereas thymol turbidity in the five patients exposed in hospitals to blood or blood products was elevated to between 0_0 and 0 6_, the test was positive (ext 0 37) in only one of the remaining subjects.

The cholestatic jaundice was induced by methyltestosterone in two cases and by chlorpromazine in three The bilirubin levels in these individuals ranged from _4 to 12, SGPT was moderately increased and thymol turbidity normal

In 15 cases (six men and nine women) a diagnosis of

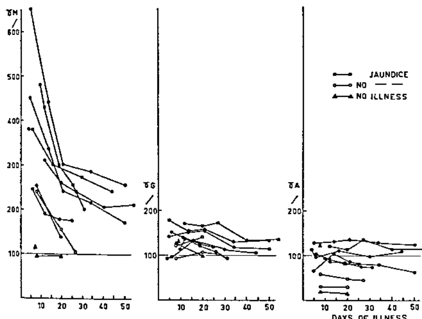


Fig 2 γG γA and γM levels in 8 cases of epidemic infectious hepatitis and in two siblings without clinical illness ●—● jaundice O—O no jaundice ▲—▲ no illness

obstructive jaundice was based on the clinical course and laboratory findings and confirmed by surgery or post mortem examination The mean age in this group was 6? years and the underlying cause proved to be malignant disease in seven and gallstone disease in eight The initial bilirubin levels ranged between 1 4 and 16 mg/100 ml the thymol turbidity was 0 02 to 0 13 the alkaline phosphatases 10 to 79 units In addition all cases had a GPT/GT ratio of less than 0 25 strongly supporting a diagnosis of obstructive jaundice (3)

RESULTS

Infectious hepatitis

The results in the sporadic cases and in the family outbreak are presented separately (Figs 1 and 2 Table I) It can be seen that in both groups all cases except one in the sporadic group who had a previous history of IH had an initial γM level above 200 / of the standard pool which fell towards normal values during the following months the most rapid decline occurring during the first four weeks The magnitude of the γM response was not age-dependent or related to the severity of the illness nor was it correlated to any of the laboratory tests except for the thymol turbidity as discussed below

In addition most cases had raised levels of γG which remained elevated for one to several months A small but significant increase in γA levels was also present initially When initial levels in 11 patients were compared to the levels in the same subjects between day 45 and 60 a significant decrease had occurred ($p < 0\ 01$)

In three cases with pronounced γM and γG responses γD levels were found to be 13 ° 65 to 55 ° and 98 to 86 of the standard pool respectively with no significant change during the course of the illness

Serum hepatitis

This group appeared to have a more heterogeneous immunoglobulin picture in that many cases especially those with a long incubation period failed to show a clearcut γM response (Fig 3) The mean γM levels initially were sig

Table I *Initial immunoglobulin levels in relation to diagnosis*

Diagnosis	No of cases	Mean levels in ±1 S D γG	γA	γM
IH sporadic	19	158±54	123±45	367±160
IH epidemic	8	142±26	88±34	376±145
SH	11	131±37	112±30	210±82
Obstructive jaundice	15	117±27	130±65	152±81
Significance of difference IH sporadic–SH		$p<0\ 2$	$p<0\ 05$	$p<0\ 01$

Fig 3 γG γA and γM levels in 11 cases of serum hepatitis O—O narcotic injection ●—● blood transfusion □—□ tattooing

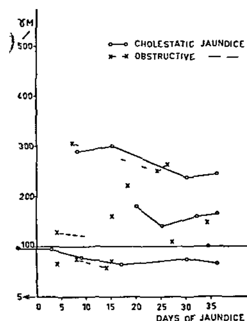


Fig 4 γM levels in 5 cases of cholestatic and 15 cases of obstructive jaundice O—O cholestatic jaundice x—x obstructive jaundice

nificantly lower than in the infectious hepatitis group ($p < 0.01$) while the γG levels were not significantly lower than in the infectious hepatitis patients ($p < 0.2$) γD levels were followed in two cases While γD was 13% throughout in one case a continuous fall from 245 to 158% occurred

Cholestatic and obstructive jaundice

In these cases the γM levels (Fig 4) were normal or high but showed no characteristic change during the course of illness The same picture was true of γG and γA levels

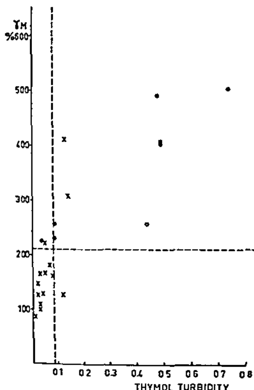


Fig 5 Correlation between thymol turbidity and γM level in sera from 57 patients Filled circles indicate sporadic and filled squares epidemic IH Open circles SH Crosses represent cases with obstructive jaundice $r=0\,76$ $p<0\,01$

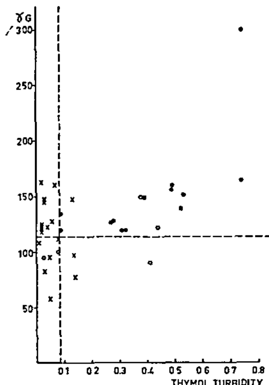


Fig 6 Correlation between thymol turbidity and γG level in 57 sera Same symbols as in Fig. 5 $r=0\,56$ $p<0\,01$

Correlation of γG and γM levels with the thymol turbidity test

A close degree of correlation between γM levels and thymol turbidity was found regardless of whether the diagnosis was IH SH or obstructive jaundice ($r=0\,76$ $p<0\,01$) Fig 5 shows the discriminatory value for differentiation of viral hepatitis from obstructive jaundice to be very similar for single γM d terminations and thymol extinction A somewhat less close correlation was found between γG levels and thymol turbidity ($r=0\,56$ $p<0\,01$) Fig 6 shows that the poorest correlation was found in the patients with obstructive jaundice who often had elevated levels of γG and normal thymol turbidities

DISCUSSION

The present study confirms earlier work regarding the immunoglobulin changes in IH the striking feature being a γM increase out of proportion to the relative increment of γG which in turn is greater than the γA reaction There is no indica tion that the magnitude of the γM response has any prognostic significance It is furthermore of interest that γM changes also occurred in unicteric cases during an IH epidemic but not in appar ently healthy contacts These changes are very similar to those found in infectious mononucleosis (23 24) and in mycoplasma pneumoniae infection (19) whereas other common virus diseases cause much smaller immunoglobulin changes (25) It is therefore reasonable to postulate that much of the immunoglobulin formed is not simply anti body directed against the invading organism per se Direct experimental testing of this hypothesis is impossible until the virus has been isolated and used in absorption exp riments but the finding of antigammaglobulin factors (6) anticytoplasmic antibodies (22) as well as antibody to liver cells (7) and monkey erythrocyte agglutinins (13) indi cate that reactions against altered host tissues may

constitute the bulk of the formed immunoglobulins The limited number of analyses of γD in IH and SH indicate that this immunoglobulin plays only a minor role in the host reaction in viral hepatitis similar observations were made by Bachmann (4) in infectious mononucleosis

Whereas only one patient in the IH series failed to show a characteristic γM or γG response this occurred in half of the SH patients The remaining half of the SH patients show immunoglobulin levels more or less resembling those typical of IH In view of the correlations found between γM and γG levels and thymol turbidity it is interesting that Neefe (18) and Green (10) found lower thymol extinctions in SH than in IH and the form of SH that prevails among drug addicts in Stockholm usually has rather low thymol turbidities (20) The much debated question whether IH and SH are caused by different agents or by different routes of administering the same agent seems to have been settled in favor of the first alternative after the carefully controlled epidemiological study by Krugmans et al (14) in an institution for mentally retarded children Our results fit well with the two-agent hypothesis especially since both agents as shown by Krugman et al (14) can be infective both orally and parentally

In the small series of cholestatic jaundice the immunoglobulins seemed unaffected indicating that liver damage in itself does not constitute an antigenic stimulus in the organism The same conclusions can be derived from the absence of alterations induced by obstruc of the biliary flow

The close agreement in this material between γM levels and thymol turbidity confirms the in vitro experiments of Hartmann et al (11) with addition of pure γM to normal serum In our material sera with marked elevation of the thymol turbidity and relatively low γM usually had markedly elevated γG Conversely marked elevation of γM with no elevation of the thymol turbidity occurred mainly in cases with obstructive jaundice in which the elevated a_1–a globulins are known to inhibit the turbidity test (1) The practical consequence is that the thymol test can serve as a fairly reliable indicator of the γM level in acute jaundice in previously healthy individuals.

The suggested inverse correlation between incubation time and initial γM raises the question whether in the cases with a long incubation period higher γM levels would have been found earlier in the course Evidence from Krugman s et al paper however is opposed to this possibility since the thymol turbidity was followed during the incubation period in both types of hepatitis and only increased synchronously with the bilirubin and SGOT

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THE EFFECT OF DEXTRAN ON PLASMA LIPIDS

A Study on Patients with Essential Hypercholesterolemia or Hyperlipemia

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Abstract In four patients with essential hypercholesterolemia and five patients with essential hypertriglyceridemia (hyperlipemia) plasma cholesterol phospholipids and triglycerides were analysed before during and after the treatment with intravenously or orally administered dextran

These plasma lipid level were lowered after dextran administered intravenously but were not influenced by orally administered dextran of higher average molecular weight which caused a profound effect in vitro

A lowering effect was observed already during the dextran infusion and persisted for 3–7 weeks A rebound phenomenon was observed 3–7 weeks after the treatment

The observations suggest that dextran influences the plasma lipid level mainly by altering the physical state of lipoproteins.

In 1954 Mollison (10) observed that dextran intravenously administered lowered the plasma cholesterol level This observation was confirmed in 1961 by Lusztig et al (9) These authors also reported (11) that dextran administration decreased the β lipoprotein level and increased the α lipoprotein level but did not influence the phospholipid level

Recently Flotte in preliminary communications (4 5) further confirmed the cholesterol lowering effect of intravenously administered dextran and also reported a similar effect of orally administered dextran with a higher average molecular weight (5)

In the present study the lipid lowering effect of intravenously administered dextran has been further investigated The levels of cholesterol phospholipids and triglycerides in plasma were analyzed at different times after intravenous dextran administration to patients with hyperlipemia or hypercholesterolemia In some patients similar analyses were made after oral administration of a dextran of higher average molecular weight

MATERIAL AND METHODS

Four patients with essential hereditary hypercholesterolemia and six patients with hyperlipemia were studied Clinical data and the initial plasma lipid values are given in Table I Patient no 9 with higher phospholipid level than cholesterol level shows no symptoms on disturbed liver function The patients had been under observation for 1–6 years At the start of the present study they had been on a low fat diet for at least six months This diet included less than 30 calories per cent from fat and was rich in polyunsaturated fatty acids In the investigation of the initial effect of intravenously administered dextran one apparently healthy male individual aged 70 years was included

The methods used for blood sampling preparation of the plasma lipid extract and the determination of total cholesterol phospholipids triglycerides and free fatty acids (FFA) have been previously described (2 13)

Procedure

Intravenous administration of dextran Macrodex® with a mean molecular weight ($\bar{M}_w$) of 70 000 was used in a 6 (w/v) solution containing 5 (w/v) glucose The short time effect of this administration was studied in cases 1 8 and 9 and in the healthy individual 1500 ml of dextran was given during six hours Blood sampling was performed in the morning before the dextran administration and then 3 8 24 and 32 or 48 hours after the administration had started The duration of the effect of dextran administration was investigated in patients 1–5 Patients 1–4 received a single infusion of 1500 ml dextran within six hours Patient 5 received 1000 ml daily for three consecutive days Fasting blood samples were drawn before the infusions started, and then repeatedly for 3–9 weeks

Peroral administration of dextran Dextran (supplied by Pharmacia Uppsala, Sweden) with a $\bar{M}_w$ of 250 000 was used in a 25% (w/v) solution Patient 3 received 15 ml twice daily for five weeks whereupon the dose

Table I *Clinical data*

Pat no	Age (y)	Sex	Ang pect	Myocard infarct	Xanthoma	Xanthelasma	Initial plasma lipids values Chol. (mg/100 ml)	P lip (mg/100 ml)	Triglyc (mg/100 ml)
1	47	♂	+				330	318	268
2	38	♂	+		+		604	472	180
3	57	♀	+	+	+	+	404	339	77
4	61	♂	+	+	+		420	370	207
5	50	♂	+	+	+	+	420	295	41
6	41	♀	+		+	+	477	383	67
7	41	♀	+			+	489	354	75
8	53	♀	+				405	330	245
9	52	♀	+				333	455	1521
10	63	♀					491	460	247

Patients no 3 5 6 and 7 have hereditary primary hypercholesterolemia

was increased to 40 ml twice daily for six weeks Patients 4–6 received 40 ml twice daily for seven weeks whereupon the dose was doubled for 5–8 weeks Patient 7 received 80 ml twice daily for six weeks

The patients tolerated the dextran administration well and no side effects were observed

The effect of dextran in vitro on the plasma lipids was compared using blood from two healthy individuals and from two patients with hyperlipemia To 8 or 10 ml of heparinized blood Macrodex the higher average molecular weight dextran or saline was added to a final concentration of 10 per cent (v/v) After standing at room temperature for 30 min the blood was centrifuged at 4 C and 1900 × g for 10 min and lipid analyses p rformed

RESULTS

Intravenous administration of dextran in the hyperlipemic patients caused a rapid decrease in the plasma lipids The effect was already apparent after three hours (Fig 1) In the healthy subject the effect of dextran was much lower During the first eight hours no change in the triglyceride level was observed but the levels of cholesterol and of phospholipids showed a slight decrease After 24 hours however all three lipid fractions were slightly lower than before

Investigation of the duration of the effect showed (Figs 2–3) that dextran caused a significant decrease in plasma cholesterol ($p < 0\ 01$) and phospholipids ($p < 0\ 01$) during the first week Thereafter these levels gradually increased and the initial cholesterol values were regained about three weeks after treatment in three patients and after about seven weeks in another patient In the patients investigated for more than four weeks the cholesterol and phospholipid levels later rose markedly above the pre experimental levels (Fig 3)

In the patients investigated during several weeks dextran administration did not clearly in

Table II *In vitro effect of dextrans*

	Added with (10 v/v)	Chol (mg/100 ml plasma)	P lip (mg/100 ml plasma)	Triglyc (mg/100 ml plasma)	FFA (mM plasma)
Postabsorptive normal blood	Saline	193	208	110	0 3
	Dextran $\overline{M}_w$ 250 000	185	204	93	0 21
Postabsorptive hyperlipemic blood (pat no 9)	Saline	425	424	1240	0 27
	Dextran $\overline{M}_w$ 250 000	176	214	448	0 27
Postalimentary normal blood	Saline	231	230	90	0 64
	Macrodex	250	242	92	0 73
	Dextran $\overline{M}_w$ 250 000	99	134	38	0 64
Postalimentary hyperlipemic blood (pat no 10)	Saline	451	408	220	0 10
	Macrodex	490	438	236	0 11
	Dextran $\overline{M}_w$ 250 000	323	325	162	0 09

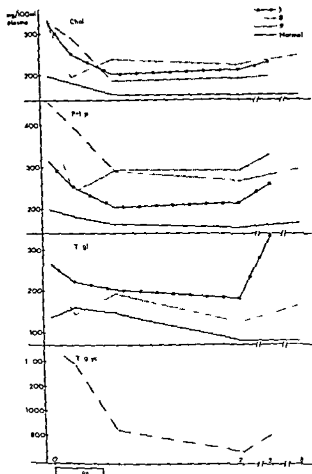


Fig 1 Plasma cholesterol phospholipids and triglycerides during the first 48 hours after an intravenous administration of dextran

fluence the normal triglyceride levels in the two patients with essential hypercholesterolemia but in the three patients with hyperlipemia a decrease was noted in all cases (Fig 2)

Per oral administration of dextran did not significantly influence the plasma cholesterol phospholipid or triglyceride levels (Table III) These studies also included the analyses of FFA These showed no changes which could be related to the dextran administration

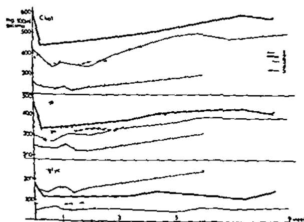


Fig 2 Plasma cholesterol phospholipids and triglycerides after an intravenous administration of dextran In patient no 2 analyses after 11 weeks showed a cholesterol value of 698 mg 100 ml plasma and a phospholipid value of 5 7 mg/100 ml plasma.

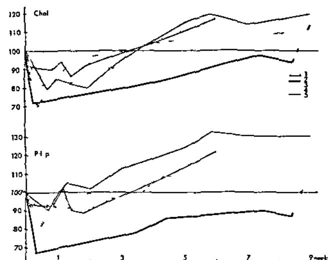


Fig 3 Plasma cholesterol and phospholipids after an intravenous administration of dextran Changes in per cent of the initial values Values for patient no 2 showed an increase for cholesterol to 118% and for phospholipids to 115% of the initial value 11 weeks after the infusion

The hemoglobin concentration the hematocrit values and the blood glucose level were not significantly influenced by dextran administration in the analyses later than 48 hours after the infusion

The *addition* of Macrodex in vitro to the blood samples caused in some experiments a slight increase in the plasma lipid values both in blood from a healthy individual and in a hyperlipemic blood presumably effects of the binding of water to the sedimented dextran The dextran with higher average molecular weight caused a marked decrease in the plasma lipid values in the blood from patients with hyperlipemia In blood from the healthy individuals a lowering effect was observed in the blood taken in a post alimentary phase but no changes were induced in the blood taken in a post absorptive phase The FFA fraction was not lowered in any of the blood samples The addition of the dextran with higher average molecular weight to hyperlipemic blood gave rise to a white layer on the top of the red cell corpuscle column after the centrifugation but no similar change was observed with normal blood

Table III *Plasma lipids during peroral treatment with dextran*

Pat no	Amount dextran (ml/day)	Period of treatment (weeks)	Analyses (*n*)	Phospholipids (mg/100 ml)		Cholsterol (mg 100/ml		Triglycerides (mg 100/ml)		FFA (m*M*)	
				M	S E	*M*	S E	*M*	S E	*M*	S E
3	—	—	3	354	12 3	403	14 1	112	15 4	0 19	—
	30	5	8	359	8 8	395	15 1	113	11 7	0 18	0 03
	80	6	8	382	6 7	450	9 9	89	4 2	0 ?2	0 04
4	—	—	3	421	11 5	491	17 3	285	10 5	0 55	0 05
	80	7	9	407	3 8	480	14 5	237	11 4	0 37	0 04
	160	9	4	386	13 1	465	32 1	406	26 9	0 40	0 06
5	—	—	3	407	20 1	537	27 5	62	3 3	0 90	0 09
	80	7	9	408	12 8	546	15 2	74	4 9	0 78	0 07
	160	6	4	409	9 1	588	8 8	85	2 3	0 73	0 14
6	—	—	3	361	13 9	427	?9 0	61	7 0	0 68	0 4
	80	7	5	387	14 0	494	20 2	56	5 3	0 59	0 09
	160	6	4	420	9 5	543	6 5	54	3 1	0 50	0 04
7	—	—	4	337	8 2	476	7 3	86	7 5	0 62	0 07
	160	6	6	357	10 2	508	17 6	87	8 0	0 52	0 09

DISCUSSION

The present study confirms the previous observation that intravenous administration of dextran causes a decrease in the plasma cholesterol level Contrary to Lusztig et al (9) we also observed a significant lowering of the phospholipid level as did Flotte and Buxton (4) The present results indicate that dextran influences all the plasma lipid fractions included in lipoprotein aggregates The plasma lipids were influenced mainly in patients with initially high values

The previously predicted effect of orally administered dextran of higher average molecular weight could not be confirmed with the dextran used in the present study

The mechanism responsible for this plasma lipid lowering effect of intravenously administered dextran is not known It has been shown previously that there is an interaction between polysaccharides and such other macromolecules as for example proteins (8) An interaction between dextrans and lipoproteins has also been observed in vitro (6 12) The degree of interaction was found to be correlated with the molecular size i e dextrans with higher molecular weights were more potent in this respect (12) It has also been shown that dextran sulfate interacts in vitro with lipoproteins (1 7)

The present in vitro studies are in agreement with these results The present observation that in hyperlipemia a plasma lipid lowering effect appears already during the dextran infusion indicates that such an interaction also occurs in vivo If a precipitation of lipoproteins really occurs in vivo an accumulation of these precipitates in the reticuloendothelial system could be expected as proposed by Iverius and Laurent (personal communication)

In the short time studies as well as in the *in vitro studies* the effect of dextran seemed to be different in subjects with different lipoprotein patterns. It is intended to follow up this finding in future studies

Clinical experience indicates that dextran administration can be given for at least shorter periods of time without disadvantages The present observation of a rebound phenomenon indicates that if dextran is to be used for continuous depression of the plasma lipids intravenous dextran administration must be given repeatedly with only short pauses between the administrations Until the risks of long term administration of dextran are known this substance ought not to be used generally as a plasma lipid lowering agent

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MUSCLE AND SKIN CLEARANCE OF ANTIPYRINE FROM EXERCISING ISCHEMIC LEGS BEFORE AND AFTER VASODILATING TRIALS

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Abstract In a series of patients with intermittent claudication, antipyrine labelled with iodine isotopes was deposited in the calf muscle and skin of the lower leg The disappearance rates of the tracers were utilized to evaluate changes in the regional blood flow in connection with exercise on a foot ergometer The clearance curves suggested that, besides an increase of muscle blood flow there was a gradual diversion of blood flow from the skin during exercise with a transitory period without any removal of tracer from the skin depot towards the end of exercise but particularly immediately afterwards The duration of this latter period was correlated to the degree of arterial obliterative disease in the leg and was considered useful in the diagnosis of incipient peripheral ischemia.

The ability of conventional vasodilating drugs to influence the behaviour of these clearance curves during exercise was tested in acute clinical experiments It was found that irrespective of the pharmacological mode of action, parenteral administration of the drugs used was accompanied by a significant decrease in clearance rate from the active muscles as compared with that registered during exercise of identical intensity before the therapeutic trial Simultaneously there was a tendency to a delayed elimination of the skin tracer The deleterious effect of the drugs on the clearance rates and thus presumably on the peripheral blood flow in the presence of arterial insufficiency seemed to be related to their depressive influence on the mean arterial blood pressure brought about by an extensive dilation of healthy vascular beds

Vasodilating substances are widely used in the treatment of obliterative arterial disease in the leg although their value has never been convincingly proved It has on the contrary been shown that in the presence of arterial insufficiency leg blood flow is often impaired by the administration of vasodilating drugs (8 12) The reason for this impairment is probably that the circulating blood volume is distributed according to the vascular resistance in different parts of the body and that consequently an induced vasodilation may lead to blood flow being diverted from arterially obstructed tissues to more reactive vascular beds Clinical studies of the changes in peripheral blood flow after the administration of vasodilating drugs have so far been restricted to resting or postexercise conditions In patients with intermittent claudication however a functional evaluation of the effect of therapy on muscle blood flow requires investigations during exercise The behaviour of the cutaneous blood flow in the exercising ischemic leg and the ability of vasodilating drugs to influence this vascular bed during exercise are also important clinical questions in the treatment of obliterative arterial disease

The clearance technique which is based on determinations of the rate at which regionally injected tracers are eliminated makes it possible to follow changes in peripheral blood flow during exercise and to evaluate separately and simultaneously the blood flow in different tissues e g muscle and skin The local clearance method as originally described by Kety (13) has been extensively utilized in studies of therapeutic effects in arterial obliterative disease (5 9 31 33 34)

Lassen (16) emphasized the importance of using tracers that are lipid soluble and therefore more easily diffusible than the hydrophilic ionic tracers formerly employed e g radioactive Na^+ and I^- Using ^{133}Xe the clearance method has proved to have a high validity in the evaluation of arterial insufficiency in studies of the muscle blood flow of the lower leg (1 18 19 30) However because of xenon s high solubility in lipids its clearance from a tissue is influenced also by

the fat content (20) In an attempt to avoid this source of error antipyrine considered to have a constant partition coefficient between tissue and blood irrespective of the fat content ((24) as quoted by (2)) was chosen as the indicator in the present study of muscular and cutaneous clearance curves from the lower leg

MATERIAL

The 46 patients participating in the present study were selected from a large clinical material of peripheral vascular disorders A few of the participants took part in more than one experiment but never in the same series All but two were males Their age varied between 45 and 72 years The main clinical diagnosis was intermittent claudication muscular fatigue and pains o the characteristic ischemic type could be provoked in the calf muscle by foot ergometry in every case Some patients also experienced varying degrees of numbness or pain in the foot during this type of exercise but none of them had manifest gangrene The existence of obliterative arterial disease in the leg was confirmed in every case by oscillography and digital plethysmography Arteriography was generally performed as well but only in cases considered for reconstructive surgery No patients suffered from angina pectoris or congestive heart failure and none was on vasodilating therapy at the time of the investigation

The controls were subjects in whom the clinical history and oscillography failed to give evidence of existing obliterative arterial disease in the leg. The age distribution of the controls was comparable to that of the patients

METHODS

Foot ergometry

The calf muscle exercise was performed on a foot ergometer with the patient in the supine position It consisted of a rhythmic lifting of weights by means of a depressable pedal To fit the pedalling position the exercising leg was slightly elevated with the knee in semiflexion (about 45) Special supports kept the lower leg in a fixed position during exercise thereby minimizing movement artefacts on the clearance curves The exercise paced with a metronome was performed at a rate of 40 contractions min By varying the load and the lifting distance the work intensity could be adapted to the individuals capacity which was estimated in a preliminary test An intensity was chosen that gave tiredness or pains of the ischemic type in the calf muscle but allowed continuous exercise for at least 3 min In the present series this corresponded to 10–30 kpm/min The patients were particularly instructed to relax the leg muscles during the passive return of the pedal In the studies in which the exercise was repeated there was always a resting period of one hour in between. All studies were performed at room temperature (19–23 C)

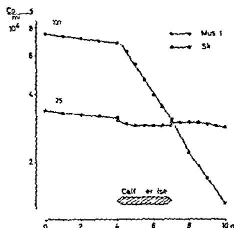


Fig 1 The clearance of I AP from muscle and skin at rest and in connection with calf exercise of an intensity high enough to give ischemic pains in the muscle and severe numbness of the foot in a patient with a femoral block and further obliterations in the lower leg arteries.

Muscle and skin clearance

The tracer was 4-iodo-antipyrine (I AP) labelled with I– or I[131] (Radiochemical Centre Amersham) diluted with saline to isotonicity The intra muscular injections of I AP (10 μC in 0 1 ml) were made into the medial head of the gastrocnemius muscle 3 cm deep into a part that from palpation seemed to take an active part in the particular exercise Equivalent amounts of tracer were used in the cutaneous depositions 5 cm proximal to the internal malleolus The needle was advanced about – cm horizontally under the skin surface before the injection was given at a depth of 1–2 mm intended to correspond to the subpapillary vascular network At least 15 seconds was spent in giving the injection and the needles (outer diam 0 4 mm) were always left in position for half a minute before retraction in order to reduce leakage of indicator With the leg adapted to the foot ergometer the disappearance rates of the indicators from muscle and skin were followed with scintillation detectors connected to a spectrometer and two ratemeters (Packard Instruments) The scintillation detectors had 2 inch NaI crystals and were placed about 10 cm above the tracer depots They were collimated to see an area 10–15 cm in diameter and the initial counting rate was (0 3 – 2) 10· c.p m. The time constant of the ratemeter was set at 3 seconds The recordings were made by means of potentiometric recorders with a paper speed of 10–17 mm/min

The linear ratemeter curves were replotted on semi logarithmic graph paper Both at rest and during exercise the logarithms of the remaining activity over the muscle depot as a function of time could generally be approximated without difficulty by a straight line (Fig 1) In some cases however the recordings during the first 3–5 min after the injection deviated from the subsequent course of the resting clearance curve probably due to injection artefacts With this part excluded the individual muscle clearance curve at rest, as well as that during

exercise could be regarded as a monoexponential function It was thus possible to express the remaining activity c at any time t as $c(0)\ e^{-kt}$ where $c(0)$ is the activity at zero time e the base of the natural logarithms and k the constant for the disappearance rate of the tracer from the muscle at rest or during exercise respectively It can be shown that this clearance constant $k = \ln 2/t\frac{1}{2}$ min where $t\frac{1}{2}$ is the half time of the actual clearance curve The methodological error involved in the graphic determination of the half times during exercise was found to be 5% in a series of 71 paired estimations from two independent plotting procedures.

The skin clearance curves at rest could also be expressed in terms of k values During exercise however the disappearance rate of the skin indicator successively decreased particularly in patients with advanced obliterative arteriosclerosis in the leg Moreover these patients often had a transitory period towards the end of the exercise or particularly immediately afterwards during which the curve ran horizontally (Fig. 1)

The background radiation varied between 0.. and 5 of the initial counting rate it was subtracted whenever more than 1% No correction could be made for arterial recirculation as both release and re-uptake of radioactivity in the diseased leg must vary individually with the degree of arterial insufficiency

Blood pressure

Thin teflon catheters were inserted percutaneously into a brachial artery The intra arterial pressure was measured by means of a pressure transducer (EMT 490 A Elema Schonander) connected to an ink jet recorder (Mingograph 4.. Elema Schonander)

Heart rate

The heart rate was determined from ECG recordings

Venous occlusion plethysmography

In some of the clearance studies the blood flow was also measured in a segmental part of one forearm, both at rest and during calf muscle exercise using a modified Dohn plethysmograph (11) The error of the method is 12.5% (26)

Oscillography

The arterial pulsations over the calves and forearms were recorded with an oscillograph (Infraton OS 3 Boucke Tubingen) The quotient between the highest amplitude of calibrated recordings from the calf and the best forearm was calculated in each case The coefficient of variation for recordings on two different occasions was 8%

Digital plethysmography

The volume pulsations of the second toe were recorded by means of a piezo-electric crystal which was in air communication with an airtight chamber enclosing the toe The recordings were made with the patient in a standardized vasodilated state arrived at by means of alcohol and indirect body heating. The amplitude of the digital pulsations was determined after volume calibration of the system The methodological error expressed as the coefficient of variation was 15–0% The pulse curves were also submitted to a shape analysis (23) to obtain information about the presence of obliterative arterial disease in the leg.

RESULTS

Muscle clearance of 1 AP

As calculated from 36 recordings the mean $t\frac{1}{2}$ of I AP injected into the gastrocnemius muscle was *at rest* 22.8 ± 17.7 min (mean $\pm$ 1 S.D.) The corresponding k value was 0.04 ± 0.02 min^{-1} The recordings were made with the patient in supine position and the leg adapted to the foot ergometer The clearance curves from the resting calf muscle in the controls did not differ significantly from those of the patients

During exercise on the foot ergometer the slope displayed by the muscle clearance curve during the rest of the exercise was mostly established within a minute If the fastest mean slope during exercise did not differ from that recorded at rest the experiment was disregarded since the tracer might have been deposited in tissue that did not actively participate in the exercise Most of these failures ($n = 10$) were among the early experiments before the importance was realized of a thorough muscle palpation as a means of finding an optimal injection site The $t\frac{1}{2}$ of the fastest mean slope during exercise was 7.2 ± 4.4 min ($n = 49$) and the corresponding k values amounted to 0.13 ± 0.07 min During exercise at the highest intensity used by the patients (30 kpm/min) the muscle clearance curves from 15 control legs had a mean k value of 0.45 ± 0.11

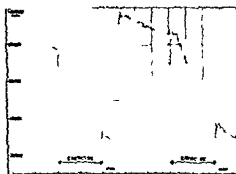


Fig. The Area clearance curves of ^{125}I AP from the calf muscle during two identical periods of exercise in a patient with a 10 cm occlusion of the left femoral artery

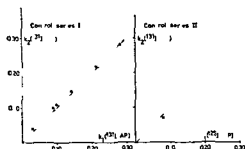


Fig 3 The relationship between the clearance constants (k and k_1) of I AP from the calf muscle as determined during two identical periods of exercise with one hour s rest in between. In control series I ^{131}I AP was the tracer in both studies while AP labelled with different iodine isotopes was used during the two periods in control series II The broken lines indicate the lines of identity which did not significantly differ from the respective regression line

min^{-1} which differed significantly from that of the patients ($p < 0\ 001$)

The reproducibility of the clearance curve from the exercising muscle was studied in 21 patients during a second identical period of exercise and after the injection of another 10 μC of I AP into the same part of the calf muscle as before the first exercise (Fig 2) In a series of 11 patients in whom the same isotope was used on both occasions the coefficient of variation for the k values was 8 0% (Fig 3) The radioactivity remaining from the first injection was not subtracted from the second clearance curve because experiments with repeated periods of exercise without any new tracer injection in between showed that the clearance curve was reaccelerated when the second exercise started thus approaching a slope similar to that of the first exercise (Fig 4) The reproducibility was not im

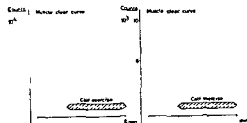


Fig 4 A comparison between the muscle clearance as registered over a single calf muscle depot of ^{131}I AP in a patient with intermittent claudication during two consecutive periods of exercise with one hour s rest in between

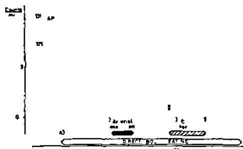


Fig 5 The effect of (*a*) body heating (*b*) transitory arterial occlusion by means of a thigh cuff and (*c*) calf exercise on the clearance curves as registered over two different skin deposits of I AP at a level 10 cm below the knee joint (O) and at the ankle (●) respectively in a patient with an occlusion of the femoral artery

proved by the use of antipyrine labelled with different iodine isotopes during the two periods of exercise the coefficient of variation in this series of ten paired k values being 11 7% (Fig 3)

After exercise most clearance curves gradually levelled off In some cases however the fast slope continued or even increased for some time after the end of exercise As there was never a steady state condition i e a monoexponential curve for more than a minute or so after exercise it was considered less reliable to calculate the $t\ ^1/_2$ or k value from this postexercise hyperemia

Skin clearance of I AP

At rest the elimination rate of the indicator (^{1} I AP) from the skin above the internal malleolus was usually slower than that of the muscle indicator For 25 out of 31 patients the mean $t\ ^1/_2$ of the skin clearance curve was 32±22 min The others had half times exceeding 100 min Fig 5 illustrates the stimulating effect of body heating upon the skin clearance curve and in contrast the complete cessation of tracer removal from the skin during a transitory period of circulatory arrest in the leg

Compared to the resting slope the skin clearance curve was in most patients found to level off during *exercise* particularly towards the end Furthermore in all but five cases there was a period immediately after exercise when the clearance curve had a horisontal course indicating a temporary halt in the removal of injected indicator from the skin elimination started again after 0 5–3 5 min (Fig 1) The pattern of the skin

clearance curves during and after exercise showed good individual reproducibility.

This delay of the clearance rate after exercise was particularly pronounced in patients who obviously had severe ischemic symptoms from the peripheral parts of the leg during and after exercise, e.g. pallor, coldness and numbness of the foot, in addition to the pains in the calf muscles. On the basis of such symptoms nine cases were distinguished from the 22 others with isolated muscular pains during the calf exercise. It was found that the two groups differed significantly in the duration of the period after exercise when there was practically no removal of tracer from the skin at the ankle. The group with severe ischemic symptoms thus had a mean duration of 2.1 ± 0.9 min compared with 0.8 ± 0.6 in the other group ($p < 0.001$). The two groups also differed clinically as evaluated by oscillography and digital plethysmography. The oscillographic quotient calf/forearm was thus lower in the group with severe ischemic symptoms than in the other group (means 0.19 ± 0.06 and 0.35 ± 0.16 respectively, $p < 0.001$). Similarly there was a difference in the size of the arterial pulsations in the toes (means 0.6 ± 0.6 and 2.3 ± 2.3 mm^3 respectively, $p < 0.01$).

In the controls on the other hand skin clearance as registered over the ankle was essentially uninfluenced by calf exercise of an intensity comparable to that used in the present study. This is consistent with earlier observations in the normal forearm (32).

Effects of Pharmacological Vasodilation

In 28 patients with intermittent claudication the removal rate of I AP from deposits in muscle and skin of the lower leg was studied during exercise of identical intensity before and after the administration of two types of conventional vasodilating drugs. Complamin®, a theophyllamine derivate of nicotinic acid, was chosen as a representative of drugs with a predominant effect on skin blood flow, while nylidrin hydrochloride (Dilatropon®) with its β stimulating action was presumed to promote blood flow of skeletal muscle in particular (10). (The drugs were supplied by AB Tika, Umeå, and AB Draco, Lund, Sweden, respectively.)

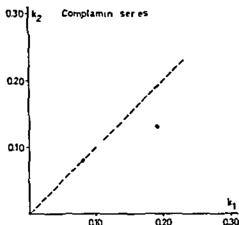


Fig. 6. The relation between the clearance constants of the intramuscular tracer during calf exercise of comparable intensity before (k_1) and after (k_2) the administration of 300 mg Complamin i.v. in nine cases of intermittent claudication. The broken line represents the line of identity.

Complamin series

After an initial determination of the skin and muscle clearance of I AP from the lower leg during exercise on the foot ergometer, nine of the patients were given 300 mg Complamin intravenously. Before the exercise was repeated another 10 μC of ^{131}I AP was added to the intramuscular depot; the skin depot was generally not refilled. Scintillation detecting was started 5 min before the injection of Complamin and was then left running continuously. The exercise was not initiated until the patient was markedly flushed, which occurred within 3 to 8 min. This sign of cutaneous vasodilation, particularly affecting the face, chest and hands, was at rest associated in three cases with an increase in the removal rate of the indicator injected intracutaneously above the internal malleolus of the diseased leg. In the other six cases the skin clearance curves were not influenced at rest by the Complamin injection. The clearance rate of the intramuscular depot was decreased in four cases and essentially unchanged in the others.

During exercise the skin clearance curve was usually less steep after the administration of Complamin than before. The immediate postexercise period without any removal of indicator from the skin was prolonged in five of the seven cases in which it could be determined accurately, with mean values of 1.4 ± 1.2 and 2.1 ± 1.1 min re-

spectively ($p < 0.05$) The clearance curve recorded over the intramuscular depots of I AP was usually found to have a longer $t\ ^1/_2$ during exercise after the Complamin injection than at comparable working intensity before the means for the nine cases being 8.0 ± 4.6 and 6.2 ± 3.0 min respectively The mean clearance constant was 0.14 ± 0.06 min^{-1} during exercise before Complamin and 0.11 ± 0.05 min^{-1} during the second exercise period (Fig 6) The mean percentage difference of the paired k values (−20.1%) was significantly distinguished ($p < 0.001$) from that observed during repeated periods of exercise in the control series Three patients with particularly advanced arterial insufficiency of the legs could not manage the same amount of work on the foot ergometer after the induced vasodilation as before This was due to the earlier occurrence of more severe ischemic pains in the exercising muscles and the elevated foot The blood pressure reaction was not followed in this series of patients but in a preliminary study on a comparable clinical material ($n = 14$) the same dosage of Complamin was found to decrease the arterial mean blood pressure by an average of 10% during the present type of exercise

Nylidrin series

Nylidrin hydrochloride (7.5 mg) was given to 19 patients as an intramuscular injection In this series too the calf muscle was relabelled with ^{131}I AP before the second exercise The exercise on the foot ergometer was started about 15 min after the administration of nylidrin A slight flush reaction was observed in a few cases but the most obvious effects of the drug were an increase in the heart rate and a decrease in the arterial blood pressure As determined at rest 10–15 min after the injection the heart rate was thus raised from 77 ± 13 to 86 ± 15 beats/min ($p < 0.001$) while the arterial mean blood pressure fell from 102 ± 9 to 91 ± 8 mm Hg ($p < 0.001$) The vasodilating effect of nylidrin on resting skeletal muscle was confirmed by the significant decrease in the vascular resistance within a muscular segment of the forearm as estimated from the arterial mean blood pressure and the blood flow determined with venous occlusion plethysmography (Fig 7) The elimination rate of ^{131}I AP from the resting calf muscle of the diseased leg was increased as often as it was decreased by the

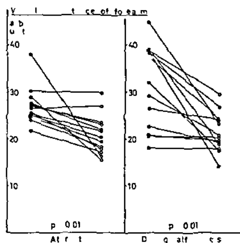


Fig 7 The effect of the nylidrin injections (7.5 mg i.m) on the calculated vascular resistance of a resting forearm segment before and during calf exercise in 13 patients with intermittent claudication Filled circles represent observations before and unfilled circles observations after the nylidrin injections

administration of nylidrin and the mean k values of the two resting periods turned out to be practically identical (0.04 ± 0.02 and 0.04 ± 0.03 min^{-1} respectively) Nor was the mean clearance of ^{125}I AP from the skin at rest significantly changed after the nylidrin injection

During exercise on the foot ergometer the arterial mean blood pressure was significantly lower after nylidrin than at identical working intensities before (means 107 ± 12 and 118 ± 12 respectively $p < 0.001$) The heart rate was si

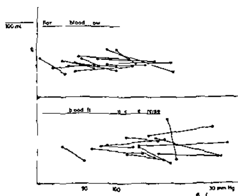


Fig 8 The independence of the blood flow in a muscular forearm segment at rest as well as during calf exercise on changes in the mean arterial blood pressure induced by nylidrin in 13 patients with intermittent claudication Symbols as in Fig 7

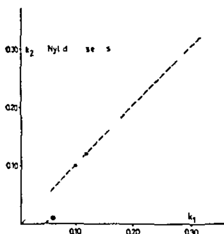


Fig. 9. The relation between the clearance constants of the intramuscular tracer during calf exercise of comparable intensity before (k_1) and after (k_2) the administration of 7.5 mg nylidrin i.m. in 19 cases of intermittent claudication. The broken line represents the line of identity.

multaneously increased from a mean of 91 ± 15 to 100 ± 14 beats/min ($p < 0.001$). As at rest, the blood flow to the forearm during the calf muscle exercise was essentially unchanged by the induced vasodilation (Fig. 8), while simultaneously there was a significant decrease in the calculated vascular resistance of the forearm (Fig. 7).

The elimination rate of ^{131}I AP from the calf muscle during exercise was retarded in most cases after the nylidrin injection. The mean $t\ ^1/_2$ was thus 7.1 ± 3.4 min during the first period of exercise compared with 11.6 ± 8.9 min during the second one. The clearance constants were 0.13 ± 0.07 and 0.09 ± 0.07 min^{-1} respectively (Fig. 9) corresponding to a mean difference of −31.1%. This result differed significantly ($p < 0.001$) from the mean difference between paired k values found during repeated periods of exercise in the control series. In six out of the ten cases in which the skin clearance curves during exercise were interpretable, the slope was less steep after nylidrin than before, being unchanged in the other cases. The transitory period after exercise without any removal of indicator from the skin was studied in 12 cases and found to be significantly increased after the administration of nylidrin (means 0.6 ± 0.8 and 1.3 ± 0.8 min, respectively, $p < 0.01$).

In this series, too, some patients had an earlier onset of the ischemic symptoms in the exercising leg after the vasodilating procedure than at comparable working intensity before.

DISCUSSION

In studies on diffusion kinetics in isolated hind legs of the cat, Renkin (25) has shown that the diffusibility of antipyrine from blood into well perfused tissue compartments is limited only by the regional blood flow. The removal rate of antipyrine from a local deposit within a tissue might on the other hand be influenced by other factors as well. One must thus consider not only the possibility of injection artefacts, including the risk of the tracer being deposited in a non-representative part of the tissue, but also a gradual increase in the mean diffusion distance for the tracer to the perfusing blood vessels. The mono-exponential shape of the I AP elimination curves obtained during the present type of leg work in the patients with arterial insufficiency indicates that under these circumstances the initial removal rate of the tracer was constant. During prolonged exercise of the forearm in normal subjects, on the other hand, Linde and Wahren (21) found a bi-exponential configuration of the muscular I AP clearance curves. An error which is difficult to avoid is the probable existence of detached isotopic iodide. The contamination of the tracer with free iodide from radiolysis was minimized by using fresh solutions of I AP. It cannot be excluded that a further enzymatic deiodination of I AP occurs within the injected tissue (28). However, the possible influence of free iodides on the clearance curves evidently did not prevent a reasonable reproducibility.

The elimination rate of a freely diffusable tracer from skeletal muscle has been used for quantitative calculation of the muscle blood flow according to the formula $\lambda \cdot k \cdot 100$ ml/100 g · min, where λ is the tissue blood partition coefficient for the particular tracer and k is the clearance constant (17). For heart muscle the λ of I AP has been found to be close to 1 (15). If this is assumed to be valid also for skeletal muscle, the mean blood flow of the exercising calf muscle at the highest intensity tolerated would have been 13 ± 7 ml/100 g · min in the patient group. This value seems to be in good agreement with the results of a similar study by Tønnesen (30)

using xenon clearance The reproducibility of the clearance of I AP from the exercising muscle was somewhat higher than has been reported with the xenon technique (1 30) This may indicate a less marked influence on the wash out curves of antipyrine from variations in the fat content within the calf muscle Good reproducibility may also require that the clearance is determined during exercise of an intensity that involves full mobilisation of the regional blood flow capacity Animal experiments recently performed on isolated muscle disclosed a tendency of the local clearance technique to underestimate the muscle blood flow as determined directly with a drop recorder (14) The correlation between the results of the two methods was however highly significant

The behaviour of the skin clearance curve during body heating and in connection with induced circulatory arrest (Fig 5) supports the hypothesis that changes in the cutaneous blood flow are reflected qualitatively at least by the clearance of freely diffusable tracers from the skin The skin clearance at rest was not expected to give relevant information about the degree of arterial insufficiency in the leg The decrease in skin clearance found in connection with exercise was on the other hand considered to reflect the deleterious effect on the regional skin blood flow from the competitive muscular demand upon the restricted blood flow of the exercising leg in the presence of obliterative arterial disease The transitory periods towards the end of exercise and immediately afterwards without any removal of tracer from the skin indicate that under these circumstances the skin may be totally deprived of its blood flow The fact that the removal of tracer from the skin was also delayed after exercise during body heating (Fig 5) suggests that the phenomenon is not due to an active vasoconstriction It is more likely that the diversion of blood flow from the skin is a hemodynamic consequence of the wide metabolic dilation of the muscle vascular bed during and after exercise and of the poorly developed autoregulation of skin blood flow (7)

The duration of the ischemic phase of the skin clearance curve from the leg after exercise on the foot ergometer was correlated to the clinical degree of obliterative arterial disease of the leg As evaluated from the colour reactions of the foot the ischemic postexercise period has earlier been utilized in the functional diagnosis of arterial insufficiency (22) and it has also been objectively disclosed by studies of the digital pulse amplitudes (27 35) The gradual diversion of blood flow from the skin during exercise has however not been demonstrated before The skin clearance reaction to exercise was more pronounced in distal than in proximal parts of the leg (Fig 5) Recordings over the foot would probably show it to be still more sensitive to the incipient stages of arterial insufficiency Owing to movement artefacts this would however require another type of detecting technique

The effectiveness of the induced vasodilation on the normal vascular bed was confirmed by a flushing of the skin or a decrease in the vascular resistance of the forearm muscles depending upon the pharmacological mode of action of the two drugs used in the respective series of experiments Within the resting ischemic leg on the other hand an increased clearance rate of the injected tracers was observed in only a few cases after administration of the vasodilating drugs During the present type of exercise the clearance rate of the muscular tracer decreased significantly after both types of pharmacological vasodilation presumably reflecting a corresponding impairment of the blood flow in the exercising muscles The cutaneous blood flow was similarly decreased after administration of the drugs as evidenced by the significantly prolonged postexercise periods of skin ischemia in both patient groups

It is reasonable to explain the unfavourable effect of the drugs upon the blood flow of the ischemic leg in terms of their general depressing effect on the arterial blood pressure secondary to the dilation of extensive vascular beds From the present study it is obvious that a general vasodilation is particularly deleterious to ischemic legs during exercise at least when performed at an intensity close to maximal capacity Under such circumstances one cannot expect that a decrease in the arterial perfusion pressure will be compensated for by a further reduction in the vascular resistance of the exercising muscles since these are probably maximally vasodilated already for local metabolic reasons The decrease in muscle blood flow during exercise after induced vasodilation was however proportionally greater than the decline in blood pressure This is probably due mainly to the phenomenon of proximal stealing, implying that the exercising muscles are partly

deprived of their blood flow because of vasodilation in tissues situated more proximally within the leg but still below the main arterial obstruction It is further possible that the arterial blood pressure in some regions distal to the main obstruction has passed the borderline of critical closing pressure (4) The major importance of the arterial blood pressure reaction for the effect of vasodilating therapy upon the blood flow through contracting skeletal muscles has earlier been elucidated in animal experiments (3 6 29)

The present finding of an impairment of both muscle and skin blood flow within a leg with restricted arterial capacity after the administration of hypotensive doses of vasodilating drugs does of course not exclude the possibility of a favourable effect of long term treatment with more cautious prescriptions We must then presuppose that an increased mean blood flow stimulates the growth and development of the critical collaterals This possibility can be investigated only by means of large clinical materials including carefully matched control series as the functional state in peripheral obliterative diseases is characterized by large spontaneous fluctuations

ACKNOWLEDGEMENTS

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ADDENDUM

Since the preparation of the manuscript the value of I AP as a tracer in local clearance studies has been confirmed by Lindbjerg (Scand J clin Lab Invest 19 1 0 1967) The errors connected with the radiochemical instability of I–AP have further been emphasized by Munck and Andersen (Scand J clin Lab Invest 19 256 1967) The existence of iodide not bound to antipyrine in the injection solution however does not invalidate the qualitative changes in clearance rate observed after the vasodilating trials since the same batches of isotopic solutions were used throughout each individual experiment

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SERUM CALCIUM AND SERUM PHOSPHORUS IN URAEMIA DURING ADMINISTRATION OF SODIUM PHYTATE AND ALUMINIUM HYDROXIDE

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Abstract Serum calcium serum phosphorus urinary calcium, the percental tubular re absorption of phosphorus (TRP%) and the phosphate excretion index (PEI) were studied in 15 patients with uraemia and in ten normal persons With increasing degrees of uraemia the authors found a decreasing serum calcium a rising serum phosphorus a decreasing urinary calcium and TRP as well as a rising PEI

It should be *emphasized that patients with very slight* renal failure (creatinine clearance >40 ml/min) showed significantly lower serum phosphorus levels than did the control group that the serum calcium fell before the serum phosphorus rose as compared with the control group when the renal failure increased that the urinary calcium fell even in very mild renal failure while the TRP and PEI did not alter until the creatinine clearance fell below 40 ml/min

For the purpose of ascertaining whether the reduction of serum calcium in uraemia is due to a reduced sensitivity to the parathyroid hormone in respect of the absorption of bone or to a possible latent hypoparathyroidism in the presence of a normal serum calcium the sodium phytate test was *used (binding of calcium in* the intestine) Furthermore aluminium hydroxide was administered (binding of phosphorus in the intestinal tract) for the purpose of elucidating whether the reduced serum calcium was due to an increased serum level of phosphorus The sodium phytate test did not give evidence of a latent hypoparathyroidism or an altered sensitivity of the bones to the parathyroid hormone Administration of aluminium hydroxide revealed that serum calcium when reduced rose to almost normal values while at the same time serum phosphorus fell This indicates that the reduced serum calcium is due partially at least to the elevated serum phosphorus in these patients It was also demonstrated that even patients with mild uraemia having a normal serum phosphorus exhibited *a greater fall of* serum phosphorus than normal patients during the administration of aluminium hydroxide This indicates a reduced renal ability to preserve phosphate

In severe renal failure the content of inorganic phosphate in the serum is often elevated and the calcium concentration reduced The elevation of serum phosphorus is presumably due to reduced glomerular filtration of phosphorus with consequent retention of phosphate The cause of the reduced serum calcium has not been elucidated Originally Albright believed that the hypocalcaemia was due to the presence of hyperphosphataemia the product of the calcium and phosphate concentration being constant in the individual patient among normals and among patients with hypoparathyroidism (1) In uraemia however the findings have not been clarified For instance hypocalcaemia may occur without hyperphosphataemia (15 19) and the serum cal*cium and serum phosphorus may to a marked* extent vary independently in the same patient (21) An attempt to explain the reduced serum calcium concentration has therefore been made in other ways It has been stated for instance that frequently these patients have a reduced absorption of calcium from the intestinal canal (9 11 19) and that this may be a contributory cause

We felt that it would be of interest to look for a possible relationship between the serum calcium and serum phosphorus levels on the one hand and the degree of renal failure on the other as this has been but sparsely elucidated in the literature and to assess whether the hypocalcaemia in uraemia might be due either to a reduced sensitivity to the parathyroid hormone upon bone absorption or to hypoparathyroidism which might be latent in uraemic subjects with a normal serum level of calcium The last mentioned possibilities would be disclosed by the sodium phytate test In this test sodium phytate (Na inositol hexaphosphate) is administered by mouth and thereby cal

cium is bound in the intestinal canal as calcium phytate (12) Patients with latent or manifest hypoparathyroidism will hereby exhibit a considerable fall in serum calcium as the parathyroid glands are unable to compensate for this The same response will be seen if the sensitivity of *the bony tissue to the calcium mobilizing action* of the parathyroid hormone is reduced

In addition we wanted to investigate whether the hypocalcaemia might be due to phosphate retention This we tried to elucidate by administering aluminium hydroxide by mouth This *will bind inorganic phosphate in the intestinal* canal as complex aluminium phosphate compounds (3) In normals this gives rise to only a minor decrease in the serum phosphorus values (14 16) In uraemics with a high serum phosphorus several authors (2 10 and others) have demonstrated that administration of aluminium hydroxide through some length of time may materially lower the serum phosphorus and attempts have been made to utilize this therapeutically

MATERIAL AND METHOD

The material comprised ten patients without signs of renal or endocrine disease having a 3-day creatinine clearance exceeding 67 ml/min and 15 patients with varying degrees of renal failure all suffering from chronic pyelonephritis Of these patients five had a creatinine clearance exceeding 40 ml/min five between 40 and 20 ml/min and five below 20 ml/min None exhibited definite radiological changes of the bones and the serum albumin concentration was normal except in one who had a creatinine clearance of 1 8 ml/min This patient had pronounced halisteresis her serum calcium was 4 55 mEq/l and serum phosphorus 1 93 millimol/l (Fig 4) The serum albumin was reduced to 2 7 g/100 ml

The experimental subjects were given the ordinary hospital diet but without milk, water or cheese in an attempt to avoid major variations in the supply of calcium and phosphorus From the 1st to the 4th day the serum calcium and serum phosphorus were determined daily by the EDTA murexide method (??) (coefficient of variation 1 5 %) and by the method of Muller (13) (coefficient of variation 3) Moreover the calcium level in the 24-hour urine was determined On the fourth day we also determined the percental renal tubular reabsorption of phosphorus (TRP %) and the phosphate excretion index (PEI) by a technique described previously (6 15) over two 4-hour periods (9 a m to 1 p.m and 1 p m to 5 p.m)

$$\text{TRP} \quad 1 - \frac{\text{phosphate clearance}}{\text{creatinine clearance}} \times 100 \qquad \text{and}$$

$$\text{PEI} \quad \frac{\text{phosphate clearance}}{\text{creatinine clearance}} - (0\,055 \times \text{serum phosphorus (in mg/100 ml)}) - 0\,07)$$ Normal values 83–93 and −0 14 − +0 14 (twice the s D)

The TRP is reported to be reduced in hyper and elevated in hypoparathyroidism The reverse applies to the PEI (6 7 15 and others)

From the 5th to the 7th day the patients received daily 9 g sodium phytate by mouth and the serum calcium and phosphorus as well as the urinary calcium were determined daily On the 7th day the TRP and PEI were again determined The 8th–14th day was a control period and from the 15th–20th day 90 ml magnesium free 6 aluminium hydroxide gel (Aludrox Wyeth) was administered daily In addition to the continued daily determinations of serum calcium serum phosphorus, and urinary calcium the TRP and PEI were again determined on the 14th and 20th days The mean values for serum calcium serum phosphorus and urinary excretion of calcium were calculated for each patient within each period and these values were used in assessing the experimental results

RESULTS

1 *Patients without renal failure*

The mean value of the serum calcium levels during the 1st control period for each individual patient are listed in Fig 1 During administration of phytate the mean value for all patients fell from 4 90 to 4 78 mEq/l a decrease which is just significant ($t = 2\,40$ $0\,05 > p > 0\,02$) How*ever there was no decrease to below the normal* range The serum calcium values increased again during administration of aluminium hydroxide ($t = 3\,14$ $0\,02 > p > 0\,01$) The serum phosphorus values did not change during the administration of phytate (Fig 1) while during the period on aluminium hydroxide there was a slight decrease ($t = 3\,71$ $0\,01 > p > 0\,001$) The mean values during the first control period and the changes in these values from this period to the phytate period as well as from the 2nd control period to the aluminium hydroxide period are shown in Tables I–III from which may be seen also the urinary levels of calcium the TRP % PEI and alterations therein During the administration of phytate there was a pronounced fall in the calcium excretion ($t = 5\,60$ $p < 0\,001$) and TRP % ($t = 5\,90$ $p < 0\,001$) as well as a marked increase in PEI During the administration of aluminium hydroxide the changes were reversed

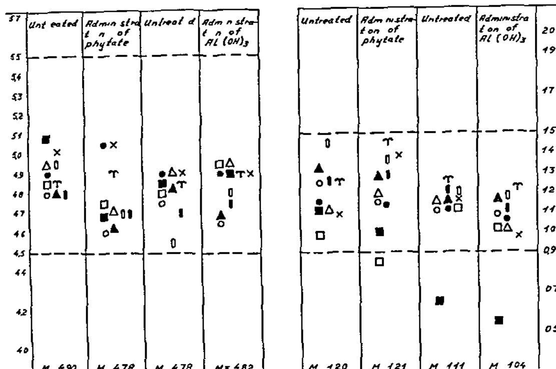


Fig 1 Serum calcium and phosphorus levels during administration of phytate and aluminium hydroxide to normals The creatinine clearance for the ten patients was as follows ■ 113 9 ml/min × 77 0 ml/min △ 107.5 ml/min 0 122.9 ml/min ● 85 2 ml min □ 96 0 ml/min Y 108 6 ml/min ○ 74 1 ml min ▲ 78 1 ml/min ▮ 81 7 ml/min

2 *Renal failure Creatinine clearance >40 ml/min*

In these patients the serum calcium levels were normal in all but one in whom they were slightly reduced (Fig 2) but did not on the whole differ from the control group ($t = 0\ 35$ $p > 0\ 1$) A look at the phosphorus levels reveals that two were below the normal range (Fig 2) On the whole the values were significantly lower than in the control group ($t = 3\ 76$ $0\ 01 > p > 0\ 001$) while TRP and PEI did not exhibit any difference The urinary levels of calcium were significantly lower than in the control group ($t = 2\ 88$ $0\ 02 > p > 0\ 01$) (Table I)

During the administration of phytate there was a fall in serum calcium similar to that in the

Table I *Mean values of some parameters before administration of sodium phytate*

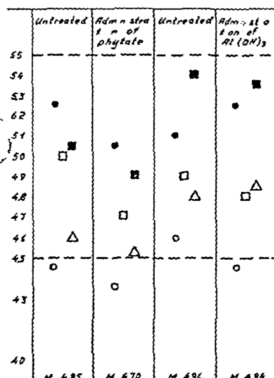
	No	Serum Ca (mEq/l)	Serum P (m mol l)	Urinary Ca (mg)	TRP*	PEI
Normal persons	10	4 90±0 09	1 0±0 14	213±52	88 5± 4 9	−0 01±0 06
Patients with renal failure	15					
Creatinine clearance >40 ml min	5	4 85±0 45	0 94±0 03	134±57	84 9± 7	+0 05±0 06
Creatinine clearance 20–40 ml min	5	4 67±0 14	1 12±0 10	89±25	73 5±14 8	−0 15±0 17
Creatinine clearance <20 ml min	5	4.29±0.3	1 93±0 08	59±41	21 9±11 6	−0 53±0 10

Table II *Changes in the mean values for some parameters during administration of sodium phytate*

	No	Serum Ca (mEq/l)	Serum P (m mol l)	Urinary Ca (mg)	TRP	PEI
Normal persons	10	−0 1?±0 17	0 01±0 14	−104±58	−10 4± 5 7	0 13±0 04
Patients with renal failure	15					
Creatinine clearance >40 ml min	5	−0 15±0 09	0 07±0 11	−45± 6	−19 1± 9 3	0 17±0 07
Creatinine clearance 20–40 ml min	5	−0 34±0 22	0 39±0 18	−30±32	−18 8±11 4	0 11±0 08
Creatinine clearance <20 ml min	5	−0 29±0 16	1 16±0 82	−8± 9	− 6 8± 5 3	−0 17±0 11

Table III *Changes in the mean values for some parameters during administration of aluminum hydroxide*

	No	Serum Ca (mEq/l)	Serum P (m mol l)	Urinary Ca (mg)	TRP	PEI
Normal persons	10	0 04±0 04	−0 07±0 06	33±11	7 0±2 6	−0 04±0 03
Patients with renal failure	15					
Creatinine clearance >40 ml/min	5	−0 0?±0 12	−0 16±0 07	−7±36	8 4±4 5	−0 04±0 06
Creatinine clearance 20–40 ml min	5	0 11±0 18	−0 27±0 16	13±28	8 0±8 0	−0 12±0 17
Creatinine clearance <20 ml min	5	0 20±0 28	−0 56±0 30	27±15	0 5±5 6	0 10±0 06



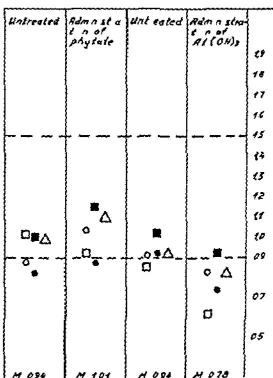


Fig Serum calcium and phosphorus levels during administrations of phytate and aluminum hydroxide to patients with renal failure having a creatinine clearance exceeding 40 ml min. The creatinine clearance for the five patients was as follows ● 44 4 ml/min ■ 50.5 ml min □ 63 0 ml min △ 66 6 ml min ○ 49 8 ml min.

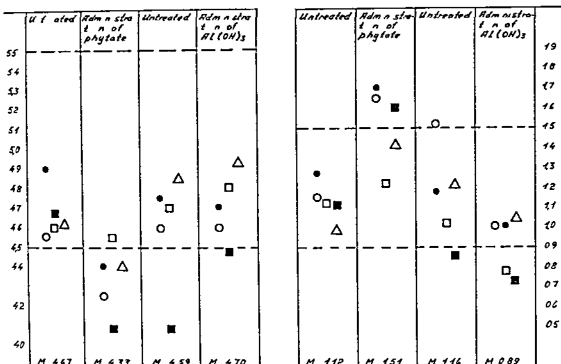


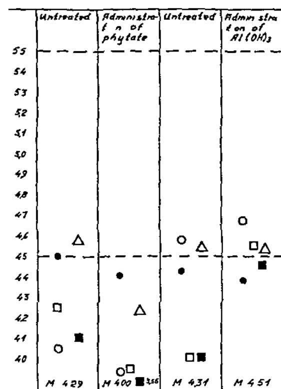
Fig 3 Serum calcium and phosphorus levels during administration of phytate and aluminium hydroxide to patients with renal failure having a creatinine clearance between 20 and 40 ml/min. The creatinine clearance for the five patients was as follows ● 29.3 ml/min ■ 37.8 ml/min △ 36.0 ml/min □ 29.1 ml/min ○ 3_.3 ml/min

control group while serum phosphorus did not alter ($t = 1.40$, $p > 0.1$). There was a pronounced fall in TRP% greater than in the control group. Correspondingly the PEI rose. During the administration of aluminium hydroxide there was no alteration in the serum calcium levels ($t = 0.37$, $p > 0.1$). On the other hand a distinct fall occurred in the serum phosphorus levels in spite of the initially low values ($t = 5.10$, $0.1 > p > 0.001$) a fall which was more marked than in the control group. The TRP% rose significantly a among the patients without renal failure ($t = 4.19$, $0.02 > p > 0.01$) (Table II and III).

3 *Creatinine clearance between 20 and 40 ml/min*

Before the administration of phytate the serum calcium levels were normal in all the subjects but the mean value was significantly lower than in the control group (Fig 3 and Table I) ($t = 3.84$, $0.01 > p > 0.001$). All the serum phosphorus values were normal and did not differ from the control group. The TRP% was significantly lower ($t = 2.98$, $0.02 > p > 0.01$) and the PEI correspondingly higher ($t = 2.87$, $0.02 > p > 0.01$).

During administration of phytate (Table II) there was a marked fall in serum calcium significantly greater than in patients without renal failure. In four subjects the values fell to below normal (Fig 3). At the same time there was a considerable improvement in the serum phosphorus values. The TRP% and PEI altered as in the previous group. During the administration of aluminium hydroxide (Table III) there was no definite change in the serum calcium values apart from a pronounced increase in one patient. On the other hand the serum phosphorus values exhibited a significant fall ($t = 3.42$, $0.05 > p > 0.02$) which was more pronounced than in the control group. The other parameters showed no definite change.



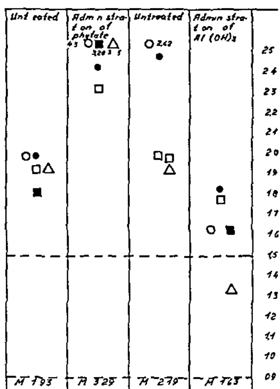


Fig 4 Serum calcium and phosphorus levels during administration of phytate and aluminium hydroxide to patients with renal failure having a creatinine clearance below 20 ml/min The creatinine clearance for the five patients was as follows △ 1 8 ml/min ● 4 8 ml/min □ 8 8 ml/min ■ 3 9 ml/min ○ 9 4 ml/min

4 *Creatinine clearance <20 ml/min*

These patients creatinine clearance was 9 4 8 8 4 8 3 9 and 1 8 ml/min In three of them the serum calcium was reduced before the administration of phytate and serum phosphorus increased in all the patients (Fig 4) During the administration of phytate there was a fall in serum calcium but for the group as a whole this did not differ from the decrease in the control group ($t = 1\ 87$ $0\ 1 > p > 0\ 05$) During the administration of aluminium hydroxide there was no definite increase in serum calcium for the group as a whole ($t = 1\ 66$ $p > 0\ 1$) The two patients with a greatly reduced serum calcium however rose almost into the normal range For serum phosphorus the findings were as follows During phytate administration there was a violent increase ($t = 3\ 16$ $0\ 05 > p > 0\ 02$) on aluminium hydroxide a pronounced fall in all cases ($t = 5\ 4$ $0\ 01 > p > 0\ 01$) greater than that in the control group and in the group with very slight renal failure However the values did not go down to the normal range ($t = 5\ 18$ $p < 0\ 001$ and $t = 2\ 94$ $0\ 02 > p > 0\ 01$)

The urinary excretion of calcium which was found to be lower than in the other patients did not alter during the administration of phytate but rose on aluminium hydroxide TRP was greatly reduced and fell even more on phytate ($t = 3\ 69$ $0\ 05 > p > 0\ 02$) After administration of aluminium hydroxide there was no change The PEI was found to be highly increased and it was found that the value fell and rose on phytate and aluminium hydroxide respectively—changes opposite to those seen in the other groups There were statistically significant differences ($t = 4\ 75$ $0\ 01 > p > 0\ 001$ and $t = 2\ 74$ $0\ 05 > p > 0\ 02$) in patients with a creatinine clearance below 20 ml/min and between 20 and 40 ml/min (Table II and III)

An attempt was made to assess the relation between serum phosphorus and serum calcium

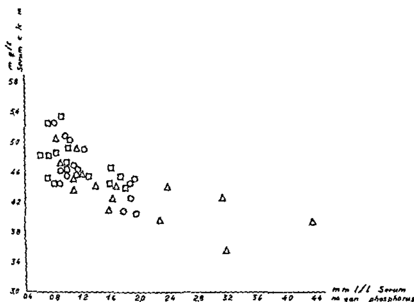


Fig 5 Relation between serum calcium and phosphorus levels before the test (○) during administration of phytate (△) and during administration of aluminium hydroxide (□)

within the entire group of uraemic subjects A correlation was found (Fig 5) high serum phosphorus being correlated with low serum calcium This correlation was most marked for untreated and phytate treated patients ($r = -0\,700$ $0\,001 < p < 0\,01$ and $r = -0\,743$ $p < 0\,001$) while it was less marked during administration of aluminium hydroxide ($r = -0\,519$ $0\,02 < p < 0\,05$)

DISCUSSION

Our main findings are as follows

Before administration of sodium phytate or aluminium hydroxide 1 the serum calcium concentration fell with increasing concentration of serum phosphorus but this fall occurred before the increase in serum phosphorus as compared with the control patients 2 the serum phosphorus levels were lower in very slight renal failure than in the control group 3 the urinary calcium fell in response to even a very slight renal failure 4 the TRP $_o$ was reduced only at creatinine clearances below 40 ml/min this has previously been reported by Gershberg (7) and by Friis (5)

During administration of phytate the normal patients and the renal patients with a creatinine clearance exceeding 40 ml/min showed a fall this fall was more marked in the group with a creatinine clearance of between 20 and 40 ml/min In this latter group however there was an increase in the serum phosphorus values presumably because phosphorus was absorbed from the administered sodium phytate a supply of phosphorus which the patients could not get rid of owing to the reduced glomerular filtration For this reason the increased drop in calcium cannot be ascribed exclusively to the calcium binding action of the sodium phytate in the intestinal canal It was a peculiar finding that the PFI fell in the patients with a creatinine clearance below 20 ml/min in contrast to the increase which occurred during administration of phytate in the other patients

On aluminium hydroxide there was an increasing fall in the serum level of phosphorus with an increasing degree of renal failure Patients with renal failure having normal serum phosphorus also exhibited a greater fall than the control group possibly because of a reduced ability of the kidneys to preserve phosphate In this connection it is worth noting that patients with a creatinine clearance below 20 ml/min increased their PFI unlike the other patients during administration of aluminium hydroxide

As already mentioned the parameters found on aluminium hydroxide were compared with the values during the 2nd control period (8th–14th day) Figs 1–4 show that these values were not always identical with those from the 1st control

period The serum calcium was significantly lower during the 2nd control period in normals ($t = 2.68$ $0.05 > p > 0.02$) and the serum phosphorus was significantly higher among subjects with severe uraemia ($t = 2.75$ $0.05 > p > 0.02$) The explanation is possibly that the sodium phytate still had some effect during the 2nd control period in these cases the values being mean values of daily determinations throughout the period If the serum calcium values on aluminium hydroxide are compared with those during the 1st control period there is no increase (Fig 1) In severe uraemia (Fig 4) aluminium hydroxide lowered the serum phosphorus levels in all patients also compared with the 1st control period and more than among normal subjects and patients with mild uraemia

In explanation of the findings it may be stated also that the reduced urinary excretion of calcium may presumably be explained on the basis of the impaired glomerular filtration of calcium This may cause a tendency to an increased serum calcium giving rise to secondary hypoparathyroidism The low serum phosphorus and the normal TRP% in the patients with very slight uraemia in our series however militate against such a possibility The low serum phosphorus might be imagined to be explicable by an increased formation of bone due to the retention of calcium and to a possibly reduced absorption of phosphorus from the intestinal canal (20) The decreasing TRP% and increasing PEI in the more severe forms of renal failure may be explained by increased parathyroid function elicited by the reduced serum calcium as emphasized by several authors (5 8 15 17 and others) though the changes may be imagined to be due to tubular damage per se

The alterations in TRP% and PEI during administration of phytate and aluminium hydroxide for normal subjects and uraemic patients with a creatinine clearance exceeding 20 ml/min accord with the fact that administration of sodium phytate (binding of calcium in the intestinal tract) stimulates and that aluminium hydroxide (binding of phosphate in the gastrointestinal tract) inhibits parathyroid function It is surprising that the behaviour of the PEI is reversed in patients with a creatinine clearance below 20 ml/min compared with the control group and with the other patients with renal failure on phytate and aluminium hydroxide but it is doubtful whether PEI is an adequate expression of the relation between the excretion of phosphorus in terms of phosphate clearance/creatinine clearance and serum phosphorus in severe uraemia in which the phosphate clearance approaches the creatinine clearance

As a final conclusion it may be stated that patients with renal failure hardly react in a way different from normals to sodium phytate as far as the serum calcium is concerned until phosphate retention occurs Judging by this finding a latent hypoparathyroidism or a reduced sensitivity to the parathyroid hormone is not present in mild degrees of renal failure The reaction to aluminium hydroxide indicates an impaired ability to preserve phosphate possibly of renal genesis even in very slight renal failure The serum calcium was not significantly affected by the administration of aluminium hydroxide when assessing the patients group by group However the hypocalcaemic patients showed a tendency to normalization of the serum calcium when assessed individually

For the uraemic group as a whole there was a negative correlation between the serum calcium and the serum phosphorus in untreated patients as well as in patients on phytate and aluminium hydroxide The findings during administration of the latter indicate to some extent that a fall in serum phosphorus tends to increase the serum calcium when the latter is reduced This is in agreement with Albright's presumption that the reduced serum calcium is due to an increased concentration of serum phosphorus provided that the serum albumin concentration is normal The phytate experiments are more difficult to assess in this respect partly because calcium is bound in the intestinal canal so that the serum level decreases and partly because the supply of phosphorus causes an increase in the serum level of phosphorus in patients with moderate to severe uraemia

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ALCOHOL INDUCED HYPOGLYCAEMIA IN CHRONIC ALCOHOLICS WITH LIVER DISEASE

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Abstract A post fasting alcohol test was performed in fourteen alcoholics with liver disease and in five healthy controls Only four alcoholics had hypoglycaemia for the same duration as that of the controls Eight of the alcoholics did not exhibit hypoglycaemia after alcohol consumption. Most of the patients who were unresponsive or less responsive to the hypoglycaemic effect of alcohol had a diabetic glucose pattern

Hypoglycaemic coma in chronic alcoholics after an alcoholic debauch is a well known condition (3 4 5 11 12 and 14) In most cases the patients have become comatose about 6–36 hours after the last ingestion of alcohol Death due to alcohol hypoglycaemia has been reported but usually hypertonic glucose injected intravenously has produced a rapid recovery (11) At first alcohol hypoglycaemia was attributed to toxic ingredients in denatured alcohol (3) However it has now been shown that hypoglycaemia can be induced by alcohol alone (2 4 5) In studying the carbohydrate metabolism in chronic alcoholics we have found that alcoholic liver disease is sometimes connected with a tendency to hypoglycaemia in other cases with diabetes (7) A diabetic glucose pattern mainly appeared in patients with liver cirrhosis while the patients with episodes of hypoglycaemia usually had fatty livers (7) In view of these findings we have tried to discover whether or not disturbances of the carbohydrate metabolism in liver disease are of importance for the occurrence of alcohol induced hypoglycaemia in chronic alcoholics

MATERIAL

The material comprised 11 men and three women of normal weight and between the ages of 31–60 (Table I) A high grade alcoholic abuse existed in all the cases and a majority of them had earlier been treated in hospital for alcoholism On the present occasion as well the reason for hospital treatment was chronic alcoholism One patient (case 1) was admitted comatose due to alcohol hypoglycaemia while the other patients were not known to have had any hypoglycaemia About two weeks of hospital treatment had preceded the examinations

Liver biopsy revealed normal liver structure in one case fatty liver in three and in ten cases fatty infiltration together with fibrous tissue (Table I) These ten cases have been classified as liver cirrhosis although the fibrosis was slight in some cases

None of the patients had oedema of the legs or ascites On admission cases 4 5 6 7 8 11 and 14 had bilirubin values between 1.5–3 mg per 100 ml At the time of the examinations the bilirubin value was less than 1 mg per 100 ml in these as in all the other cases

METHODS

Oral glucose tolerance test The patients were given 1 5 g glucose per kg body weight maximally 100 g

Intravenous tolbutamide test One g of tolbutamide in 20 ml solution was injected over a period of 2 min

Post fasting alcohol test After 44 hours fasting the patient was given 40 ml of 96% alcohol diluted with water to 15–35% A slight euphoria nearly always followed the alcohol administration, but in other respects nothing unusual was noticed and none displayed any symptoms indicating hypoglycaemia Blood glucose was estimated during an additional 4 hours fasting Glucagon (1 mg) was injected intramuscularly 3 hours after the alcohol administration A post fasting alcohol test was also performed upon five healthy persons of normal weight

Liver biopsy was carried out with a Vim Silvermann needle

Blood glucose was determined with an Auto-Analyser according to the method of Hoffman (9) Normal fasting blood glucose concentration 55–110 mg per 100 ml

RESULTS

Oral Glucose Tolerance Test (Table II)

Six patients had a diabetic glucose tolerance test with blood glucose values above 155 mg per 100

Table I *Age and sex distribution of 14 alcoholics and liver biopsy diagnosis*

Case	Age	Sex	Liver biopsy diagnosis
1	57	♂	Fatty liver
2	34	♂	Fatty liver
3	31	♂	Fatty liver
4	45	♂	Portal cirrhosis
5	44	♂	Portal cirrhosis
6	43	♂	Portal cirrhosis
7	60	♀	Portal cirrhosis
8	59	♂	Portal cirrhosis
9	54	♀	Portal cirrhosis
10	51	♀	Portal cirrhosis
11	38	♂	Portal cirrhosis
12	59	♂	Portal cirrhosis
13	55	♀	Portal cirrhosis
14	41	♂	Normal liver

ml two hours after glucose ingestion (cases 3 4 5 7 8 and 12) On several occasions case 3 had elevated fasting blood glucose values and some times glucose in the urine He was judged to have manifest diabetes

Intravenous Tolbutamide Test (Table III)

In cases 3 4 7 and 12 the blood glucose decrease 30 min after the injection was less than 25% from the fasting blood glucose concentration Persistent hypoglycaemia was noticed in cases 10 13 and 14 (Blood glucose 38 35 and 44 mg per 100 ml 2 hours after the injection of tolbutamide)

Table II *Oral glucose tolerance test*

Case	Fasting value	Minutes 30	60	90	120	150	180
1	82	148	140	9[illegible]	92	116	104
2	72	134	110	100	96	100	56
3	121	252	348	388	346	270	232
4	76	158	240	238	212	150	76
5	81	113	147	164	155	135	123
6	86	131	1[illegible]6	119	1[illegible]5	114	82
7	88	152	153	171	175	171	14[illegible]
8	104	202	232	248	213	172	125
9	108	136	112	92	96	88	68
10	80	128	116	99	91	85	47
11	75	184	153	135	98	55	42
12	78	118	156	174	168	150	118
13	75	190	165	75	60	55	65
14	53	136	141	139	79	46	42

Table III *Intravenous tolbutamide test*

Case	Fasting value	Minutes 15	30	45	60	90	120	150	180
1	76	50	32	24	32	50	60	60	60
2	82	60	40	50	56	60	72	68	—
3	100	92	80	80	72	60	64	72	72
4	92	88	76	68	64	66	68	68	64
5	79	68	57	50	47	52	57	63	63
6	87	68	48	45	39	58	62	61	6[illegible]
7	80	72	67	56	52	54	56	65	64
8	89	82	66	58	55	68	65	63	67
9	87	67	59	46	44	59	61	63	61
10	57	43	28	22	3[illegible]	34	38	40	36
11	78	66	50	41	46	46	56	62	6[illegible]
12	88	80	68	65	72	80	83	—	88
13	72	55	47	15	18	29	35	—	65
14	61	55	36	34	33	31	44	—	50

Post fasting Alcohol Test (Table IV)

Controls

All five control subjects revealed hypoglycaemia after alcohol consumption This hypoglycaemia took place ½–1 hour after the alcohol ad ministration In four cases the hypoglycaemia re mained during a 4-hour glucose determination while in the fifth case an elevation of the blood

Table IV *Post fasting alcohol test*

Cases	Fasting values	Minutes 30	60	120	150	180[a]	210	240
1	60	—	38	22	—	24	36	36
2	83	69	61	55	56	61	62	8[illegible]
3	105	—	68	50	—	6[illegible]	115	106
4	78	72	70	60	59	58	60	61
5	69	66	75	72	69	70	87	90
6	66	60	52	55	47	45	66	64
7	84	79	77	77	72	71	84	89
8	96	90	88	81	80	75	95	97
9	70	51	48	38	36	34	39	44
10	74	66	65	61	55	55	66	7[illegible]
11	82	77	71	66	66	61	77	8[illegible]
12	88	76	70	62	55	62	88	9[illegible]
13	76	66	62	45	40	48	54	54
14	39	34	30	20	18	20	22	[illegible]
Control								
1	64	52	44	32	30	[illegible]8	30	[illegible]2
2	90	59	52	46	50	52	57	57
3	98	56	54	54	54	44	4[illegible]	46
4	64	44	42	40	36	[illegible]2	4	40
5	64	58	54	44	4	40	4	48

[a] 1 mg glucagon was injected i m

glucose level to just above 55 mg per 100 ml was noticed during the last half hour of the test In none of the controls did the blood glucose response to glucagon injection exceed 10 mg per 100 ml

Patients

In four patients (cases 1 9 13 and 14) hypoglycaemia was found to persist to the end of the test In these cases the highest blood glucose rise after glucagon was 12 mg per 100 ml

Two other patients (cases 3 and 6) had hypoglycaemia which disappeared during the last half hour of the test Their blood glucose response to glucagon was respectively 53 and 21 mg per 100 ml

In the remaining eight cases (cases 2 4 5 7 8 10 11 and 12) no hypoglycaemia occurred Seven of these patients showed a blood glucose rise after glucagon amounting to between 17–30 mg per 100 ml In one of them (case 4) the response to glucagon was only 3 mg per 100 ml

DISCUSSION

Alcohol induced hypoglycaemia has been studied extensively during recent years (1 2 4 5 6 10) It has been found that in order to produce hypoglycaemia a period of fasting must precede the administration of alcohol During a period of fasting fat gradually becomes the predominant fuel According to Arky and Freinkel (2) the hypoglycaemic effect of alcohol is unmasked only after a more or less complete diversion to fat metabolism has occurred The hepatic stores of glycogen are then low and at the same time the gluconeogenesis is inhibited by alcohol (2 6) However in animal experiments it has been shown that the lack of liver glycogen combined with an inhibition of gluconeogenesis does not always produce hypoglycaemia This is due to the fact that alcohol apart from decreasing the glucose release from the liver also impairs the glucose utilization in the periphery (10) Hypoglycaemia develops provided that the glucose release from the liver diminishes more than the simultaneous decrease of the peripheral glucose utilization (10)

Arky and Freinkel (2) using a standardized alcohol infusion test have induced hypoglycaemia in 16 healthy subjects after a fast of 72 hours Field et al (4) found hypoglycaemia in five healthy individuals when alcohol was consumed after two days fasting Also in this investigation hypoglycaemia developed in five controls when alcohol was given after two days fasting In Field et al s as well as in our controls the hypoglycaemia remained during the whole period that blood glucose was determined To sum up it appears that hypoglycaemia following alcohol administration during a state of fasting is a normal reaction in man

On the other hand only four alcoholics (cases 1 9 13 and 14) had hypoglycaemia for the same duration as that of the controls Two cases (3 and 6) had transient hypoglycaemia In the remaining eight cases no hypoglycaemia was found A similar resistance to the hypoglycaemic effect of alcohol has been observed in obese subjects and in non-obese subjects treated with cortisone (2) These two conditions are often combined with a diabetic glucose pattern Five of the eight alcoholics who did not develop hypoglycaemia after alcohol consumption had diabetic glucose tolerance curves Morover three of these cases had a tolbutamide test consistent with diabetes mellitus One of the two cases with a transient hypoglycaemia had overt diabetes (case 3) Finally none of the four cases (1 9 13 and 14) with a normal post fasting alcohol test had a diabetic glucose pattern

In case 14 hypoglycaemia occurred during the post fasting alcohol test immediately before the alcohol administration Thus in this case a period of fasting alone produced hypoglycaemia The same patient had persistent hypoglycaemia after intravenous tolbutamide injection These findings are consistent with insulinoma (11) However the serum insulin level in peripheral venous blood was not elevated during the above mentioned tests which renders the diagnosis of insuloma unlikely (8)

The interpretation of the response to glucagon injection during the post fasting alcohol test must be made with caution Thus an elevation of the blood glucose during the end of the test might have occurred spontaneously and not owing to the glucagon injection However in the controls and the patients with normal post fasting alcohol test the blood glucose response to glucagon was practically absent (between 2–12 mg per 100 ml) while the other patients with the

exception of case 4 showed a blood glucose rise after glucagon amounting to 17–53 mg per 100 ml

To summarize most of the patients who were unresponsive or less responsive to the hypo glycaemic effect of alcohol had a diabetic glucose metabolism in contrast to those patients who had a "normal" hypoglycaemia after the administra tion of alcohol Thus it is possible that alcoholic liver disease with decreased glucose tolerance is connected with a resistance to the hypoglycaemic effect of alcohol Since a diabetic glucose pattern is a feature of cirrhosis (7 13) it follows that the alcoholic with cirrhosis may be less responsive to the hypoglycaemic action of alcohol

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HEMODYNAMIC EFFECTS OF β ADRENERGIC BLOCKADE IN PATIENTS WITH COMPLETE HEART BLOCK AND IMPLANTED PACEMAKER

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Abstract The hemodynamic effect of 5 mg propranolol intravenously was studied during permanent endocardial pacing in nine patients with complete atrioventricular heart block. The heart rate was constant at a preset pacemaker frequency After the injection of propranolol the cardiac output was reduced in all patients and a fall in maximal arterial *dp/dt* was found Atrial retardation occurred in all patients The atrial contractility as judged from the maximal atrial systolic pressure was not reduced. It is concluded that the reduction of cardiac performance after propranolol in patients with constant ventricular rate is a result only of the negative inotropic effect on the ventricular myocardium

β adrenergic blockade has a negative inotropic and a negative chronotropic effect on the heart (1 3) In patients with artificial pacemakers these effects of β blockade can be studied separately Such studies may also give some information about the effects of β adrenergic blockade on the atrial activity

MATERIAL AND METHODS

Nine patients with complete atrio entricular heart block were investigated during permanent pacing by endocardial electrodes. There were eight males and one female Their ages ranged from 58 to 78 years.

The patients were all in good clinical cond tion with out heart failure The ECGs showed regular pacemaker induced QRS-complexes and P waves independent of the pacemaker rhythm without competitive rhythms

The patients were investigated lying comfortably in bed with one or two pillows One PE 160 catheter was inserted percutaneously through an antecubital vein into the right atrium and another through the right femoral artery into the terminal part of the aorta The patients were allowed 15 min relaxation after the appl cation of the equipment.

Card ac output was determined by the indicator dilu tion method using injections of _5 mg indocyanine-green (Cardiogreen) into the right atrium and withdrawal of arterial blood at a constant rate (40 ml/min) through a DenNox densitometer connected to a Cambridge re corder The blood was immediately reinfused The area under the dilution curve was measured with a planimeter The system was calibrated using one blood sample with known dye concentration.

The time interval from the dye injection to the dye appearance in the densitometer was noted as circulation time Arterial and right atrial pressures were measured by Statham pressure transducers P 23 Gb and P 23 BB and recorded on an Elema Schonander Mingograph As pressure reference point was chosen a point in the fourth intercostal space 10 cm from the back of the patient Artery pressure *dp/dt* was obtained from the pressure recordings or by using a R/C differentiating circuit Pacemaker and atrial rates were calculated from the ECG

Cardiac output and circulation time were determined 15 and 5 min before and 5 15 and 30 min after a slow injection of 5 mg propranolol (1 mg/min) in the right atrium ECG intra arterial and right atrial pressures were recorded at short intervals

RESULTS

Cardiac output (Fig 1) before the injection of propranolol averaged 4 62 l/min (range 3 8–5 5 l/min) After injection it was reduced in all pa tients The average cardiac output 5 15 and 30 min after the injection was 3 87 3 87 and 3 90 l/min respectively

Circulation time (Fig 2) before the injection of propranolol averaged 16 3 sec (range 13–20 sec) After injection it was prolonged in all patients The average circulation time 5 15 and 30 min after injection was 18 1 19 0 and 19 0 respec tively

Heart rate was constant in each patient at a preset pacemaker frequency ranging from 70 to 76 (mean 72) beats/min

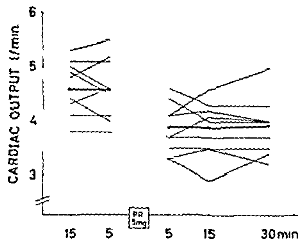


Fig 1 Effect of propranolol on cardiac output in nine patients during permanent endocardial pacing PR = time for injection of 5 mg propranolol The thick line represents the mean values

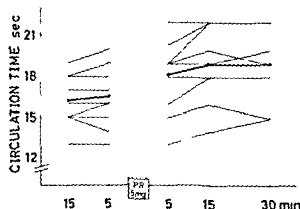


Fig Effect of propranolol on circulation time in nine patients during permanent endocardial pacing The thick line represents the mean values

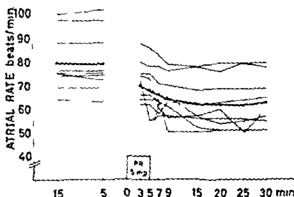


Fig 3 Effect of propranolol on atrial rate in nine patients during permanent endocardial pacing The thick line represents the mean values

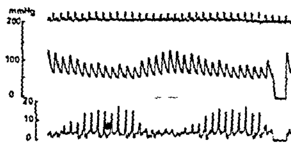


Fig 4 Illustration of pressure fluctuations due to atrioventricular asynchronism The registrations are from above ECG lead II intra arterial pressure and intra atrial pressure Ventricular rate = 76 atrial rate = 81 Paper speed 5 mm per second

Stroke volume before the injection was on an average 64 2 ml 5 15 and 30 min after the injection it was reduced to 53 8 53 8 and 54 2 ml

Atrial rate (Fig 3) was reduced from mean 79 to 63 beats/min Maximal reduction occurred 15 to 25 min after the injection

Intra-arterial pressure did not change after the injection Pressure fluctuations due to varying atrioventricular timing were seen both before and after the injection (Fig 4)

Maximal arterial pressure dp/dt before the injection ranged from 490 to 1770 (mean 1115) mm Hg per sec Fifteen min after the injection it ranged from 430 to 1670 (mean 907)

Right atrial pressure showed great fluctuations in each patient due to the atrioventricular asynchronism (Fig 4) Maximal right atrial pressure which was seen when atrial and ventricular contractions occurred simultaneously was unchanged after propranolol in three patients where such cannon waves occurred in sufficient number to be calculated Mean right atrial pressure changed slightly from an average of 5 mm Hg before to an average of 6 mm Hg after the injection of propranolol

DISCUSSION

In a previous study (1) of the hemodynamic effects of propranolol in patients with acute myocardial infarction we found 28 7% reduction of cardiac output after β adrenergic blockade Robinson et al (5) found 20% reduction in resting normals and Howitt et al (4) found 24% reduction in resting hyperthyreoid patients In pacemaker patients with fixed heart rate Donoso et al

(2) found 15° reduction of cardiac index after injection of propranolol which is in good agreement with the 16 2 reduction found in the present study In pacemaker patients the negative chronotropic effect of the drug is eliminated and the reduction is therefore caused mainly by the negative inotropic effect However one cannot eliminate a possible effect of atrial retardation on the cardiac performance

It is well known that the atrial contraction contributes to the diastolic filling of the ventricles and that the lack of timing during ventricular pacing reduces cardiac output compared to the cardiac output in sinus rhythm or during synchronized pacing (6) Certain limits for the PQ interval can be demonstrated where the effectivity of the heart is maximal (8) Slowing of the atrial rate in patients with fixed ventricular rate may therefore result in fewer atrial contractions falling within the appropriate time interval and thereby reduce cardiac output

On the other hand it is possible that atria contracting during ventricular systole not only fail to contribute to but may also impair the ventricular filling From this point of view reduction of the atrial rate should improve cardiac function These two effects of atrial slowing may possibly counteract each other and the cardiac output reduction demonstrated in this study is therefore presumed to be a result of the negative inotropic effect of the drug

The atrial contractility as judged from the maximal atrial systolic pressure was not reduced after injection of propranolol indicating no negative inotropic effect on the atrial myocardium Such an effect could have been masked by a rise in the ventricular end-diastolic and atrial diastolic pressure The atrial diastolic pressure however did not change after propranolol

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OPEN CORRECTION OF FALLOT S ANOMALY IN THREE PATIENTS OVER FORTY YEARS OF AGE

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Abstr ct The three most elderly ca.e ar p es nted in a consecutive series of 56 patients w th the anomaly of Fal lot totally corrected dur ng the period 1959 1966 The three p tients 43 55 and 56 years of ag at operation all survived All the patients were studied by pre and post operative heart catheterizat on and angiography

The complete correction of Fallot s anomaly in patients in middle life is rare because it is unusual for these patients to reach the age of 20 without undergoing surgical treatment It has been cal culated that only one out of ten patients with cyanotic congenital defects attains this age with out treatment The mortality during the first year of life is very high Fallot s anomaly is the most favourable cyanotic defect from a prognostic point of view No doubt adult patients with Fal lot s anomaly will be even more rare in the future as the congenital heart defects are more and more being investigated and treated at an early age

The essential feature in Fallot s anomaly is the combination of a large ventricular septum defect and steno is of the right ventricular outflow tra t In serious cases equilibrated ventricular pressures are obtained Three variants may be distinguished depending on the degree of stenosis If the steno is is slight there is a pure left–right shunt and these patients are therefore acyanotic If there is severe stenosis a predominant right–left shunt is ob tained and the patients are always cyanotic If finally the stenosis is moderate a combined right–left and left–right shunt is obtained These pa t ents also are cyanotic However some cases are acyanotic at rest but become cyanotic at work

The purpose of this report is to give an ac count of the three oldest patients in a series of 56 consecutive cases operated on for complete correction during the period 1959–1966 Most of the patients were cyanotic at rest and all became cyanotic du ing work All cases were operated on by V O Bjork M D using a heart lung machine and hypothermic arre t In good agree ment with previous series the high st mortality was noted amongst the youngest patients One third of the patients were less than ten years old at the time of operation but neverth less re presented two thirds of the mortality The total mortality in this material was 30 % The three oldest patients were all men and all survived the operation they were 43 55 and 56 years of age at the time of the operation

CASE REPORTS

Case 1 S A born 1921

The patient was a 43 year old clerk, who had suffered from heart disease since birth Cyanosis began at th a.e of six and increased gr atly from the age of 1? when he also began to suffer from shortness of breath and palpitation of the heart on m nor exertion He was retired with a disab ement pension s x months before the complete correction In h s youth he had a tendency to nose b'eed ng and on several occasions was also treated for bleed ng gastric ulcers Several years before the heart operation cong n tal cystic k dneys were also diagno ed In the last f w years the non prote n nitrog n had been slightly ncreased to 50 mg Complete corre t on was considered in 1959 but the operation was decl ned in iew of the kidney disease On admiss on to the hosp tal marked p r pheral cyanosis with clubbing was noted

On auscultation of the heart the first sound was normal and the se ond sou d somewhat accentuated in the th rd and fourth ntercostal spaces on the left side Over the whole precordium with a max mum paraster nally in the fourth intercostal space on the left side a grade 5 (scale 1–6) pansystolic murmur was heard No d astolic murmu could be heard

The ECG showed hypertrophy of the ight ventricle and suspected hypertrophy of the right atrium Further clinical and haemodynam c data a e shown in Table I

Ang ography from the right atr um showed enlarge ment of the atrium and the right ventricle Both in

Table I *Clinical and haemodynamic findings*

Cases		Haematocrit ( )	Working capacity (kpm/min)	X ray: Heart volume (ml/m²)	X ray: Left ventr enlargement	Right heart catheterization data: Work load (kpm/min)	Art O_2 sat. ( )	Flow data: V_O	Q_p	Q_s	L–R	R L	Pressures (mm Hg): RA_m	RV	3rd ventr	PA	BA
Case 1	Pre	70	200	760	+	Rest	49	248	1 8	9 5	0	7 7	5	130/10	48/4	9/3	120/70
43 y	op					100	33	429	—	—	—	—	—	150/20	—	22/6	110/70
	Post	55	600	580	(+)	Rest	98	—	—	—	—	—	—	14/2	—	—	110 60
	op																
Case 2	Pre	50	350	810	+	Rest	90	260	6 6	3 2	3 9	0 5	1	146/10	85/6	39/19	140,80
55 y	op					300	86	917	—	—	—	—	—	140/20	—	45/15	195/110
	Post	45	450	730	+	Rest	94	282	6 0	6 0	0	0	−1	37/1	—	21/7	140/70
	op					150	90	494	—	—	—	—	—	60/5	—	37/16	150,80
Case 3	Pre	44	700	580	+	Rest	95	267	8 6	4 7	4 4	0 5	1	135/4	—	18/11	130 65
56 y	op					400	80	1017	—	—	—	—	—	—	—	35/16	175/100
	Post	37	700	520	(+)	Rest	98	259	6 8	3 6	3 2	0	6	70/80	48/10	30/10	140/80
	op					400	96	1089	—	—	—	—	9	95/10	—	40/10	150 80

V_O = oxygen consumption (ml/min)
Q_p = pulmonary flow (l/min) (11)
Q_s = systemic flow (l/min)
L–R = left to-right shunt (l/min)
R–L = right to-left shunt (l/min)
RA_m = mean right atrial pressure (mm Hg)
RV = right ventricular pressure (mm Hg)
PA = pulmonary artery (mm Hg)
BA = brachial artery (mm Hg)

fundibular and valvular stenosis of the pulmonary valve could be demonstrated as well as a large ventricular septum defect

The laboratory tests showed that the haematocrit value was 70 *the thrombocyte count 89 000 per mm the* non protein nitrogen 50 mg ₒ and the endogenous creati nine clearance 52 4 ml per min

In December 1964 the patient was operated on A very tight infundibular stricture was resected and a commis surotomy of the pulmonary valve was performed The ventricular septum defect was closed with a dacron prosthesis measuring ? 5 × 3 5 cm It was also necessary to suture a dacron prosthesis in the outflow area of the right ventricle The patient was treated in a respirator for ̶4 hours post-operatively

On re admission 13 months after the operation the patient had improved considerably He was now working half time as a bank clerk and was no longer troubled by shortness of breath or palpitations He could walk up several stairs without discomfort a feat which had been inconceivable before the operation He was not cyanotic *and no digitalis therapy was required He had noted* transverse lines on his nails The nail tissue which emerged proximally from these De Beau lines was normal the nail growing distally of the transverse bands had the shape of a watchglass

On examination no peripheral signs of incompensation could be observed except for slight labial cyanosis There was no clubbing On auscultation the first sound was weak and the second somewhat accentuated and split With a maximum over the pulmonic area a grade 2 pan-systolic murmur was heard with an early-systolic maximum. No diastolic murmur was auscultated

The ECG showed sinus rhythm with right-sided bundle branch block and hypertrophy of the right atrium There were no signs indicating hypertrophy of the left ventricle either before or after the operation

Table I gives data from work tests X rays of the heart and heart catheterization The catheterization was carried out from the right vena femoralis and failed to reach the pulmonary artery

Angiography from the right ventricle showed a reduc tion in the volume of this ventricle but still a clear enlargement of the right atrium The outflow area of the right ventricle was just as wide as the main trunk of the pulmonary artery No inter ventricular shunt was found.

This patient has recently been examined again ?9 months after the operation He has now improved further both subjectively and objectively However there is still a slight increase of the haematocrit value

Case 2 A L born 1909

This patient was a 55 year-old man Even as a child it was noticed that he suffered from shortness of breath *and cyanosis on slight exertion and that he easily fainted.* He was released both from physical training at school and from compulsory military service on account of heart disease During the last few years before the complete correction he had chest pains as in angina pectoris and joint symptoms as in rheumatoid arthritis He was retired on a disablement pension a few months before the heart operation

On admission no dyspnoea was noted at rest There was pronounced peripheral cyanosis and clubbing. On auscultation the first sound was faint the second sound was fainter over the base than over the apex and was

probably not split Over the xiphoid area an early diastolic sound was also heard With a maximum in the fifth intercostal space parasternally on the left side a medium-pitched murmur (grade 3–4) was heard covering the greater part of systole

ECG showed a right ventricular conduction defect this did not prove ventricular hypertrophy and was possibly a sign of an old posterior wall infarction There were no signs of right atrial hypertrophy The data from the work tests, X ray photographs of the heart and heart catheterization are given in Table I

Right ventricular angiography showed enlargement of the ventricle and infundibular and valvular stenosis of the pulmonary valve Considerable dilatation of the pulmonary artery was also noted

The laboratory tests showed that the haematocrit value was 50% the serum creatinine 1.2 mg% and the thrombocyte count 115 000 per mm

Complete correction was carried out in August 1964 The infundibular stricture was resected and two incisions were made in the dome of the pulmonary valve A teflon prosthesis measuring 3 by 4 cm was sutured in the large ventricular septum defect. The pressure in the right ventricle before the correction was 115/70 mm Hg and after the correction 55/25 mm Hg Post-operative respirator treatment was necessary for eight days Afterwards the course was a smooth one though in the initial period there was a marked tendency to extrasystoles

At the post-operative examination 16 months later only a moderate improvement was noticed The patient was less troubled by shortness of breath and had noticed no cyanosis He often had attacks of coughing, which produced large quantities of yellow sputum At the examination it was noted that there was slight labial cyanosis and that clubbing of the fingers still remained though less marked On auscultation the first sound was normal and the second sound was louder over the apex than over the base of the heart Over the whole precordium a grade 3 pan systolic murmur was heard with a maximum over the apex Only with the phonocardiogram was it possible to detect a short low pitched diastolic murmur with a maximum over the apex

ECG showed sinus rhythm and right bundle branch block Table I gives the results of the heart catheterization X ray photographs of the heart and work tests

In angiography the right ventricle was still enlarged and there was now ample width of the outflow area No shunt could be demonstrated on the ventricular or atrial level.

The X ray examination of the lungs showed normal conditions but right-sided bronchography showed wall changes as in chronic bronchitis Lung function tests disclosed an obstructive limitation of the ventilatory function The haematocrit value and the results of the other routine tests were normal.

Case 3 A. K *born 1906*

This patient was a 56-year-old man who ever since childhood had readily become short of breath and had palpitations of the heart on exertion but had never become cyanotic He had had difficulties in keeping up with his playmates games Otherwise he had been fairly healthy and had even managed to do relatively heavy work in a gravel pit He came into the hospital for investigation after his health had greatly deteriorated in the last six months with marked dyspnoea on exertion and increasing tiredness

On admission the patient had dyspnoea during conversation clear cyanosis of the lips and nail bed and clubbing On auscultation a faint second sound was heard over the apex and parasternally in the first left intercostal space Over the left sternal border with maximum in the second intercostal space a harsh grade-4 pansystolic murmur was heard No diastolic murmur could be heard

ECG showed sinus rhythm marked right ventricular hypertrophy and suspected hypertrophy of the right atrium.

The data from work tests X rays of the heart and heart catheterization are shown in Table I

Angiography of the left ventricle showed a ventricular septum defect situated high up The right ventricle was sufficiently opacified to demonstrate stenosis of the pulmonary valve with post stenotic dilatation

The laboratory tests showed that the haemoglobin value was 15 5 g the haematocrit value 44 and the thrombocyte count 144 000 per mm

Complete correction was carried out in March 1963 A teflon prosthesis was inserted in the large ventricular septum defect Commissurotomy of the pulmonary valve was carried out from the pulmonary artery and an infundibulectomy was also necessary There was only an insignificant amount of tissue between the pulmonary and the aortic valves and some sutures had to be placed in the sinus of the pulmonary valve as there was no real border between the pulmonary and the aortic valves Post-operatively the patient was treated in a respirator and on several occasions had convulsive seizures which were believed to have been caused by air embolism A wound infection complicated the post-operative course and a secondary operation became necessary as the cerclage threads were cutting through the sternum

On re admission 20 months after the operation, the patient felt moderately improved compared with the period before the operation He was not fit for work and had been retired on a disablement pension At the examination no signs of peripheral incompensation and no cyanosis were noted As before clubbing was present.

On auscultation the second sound was constantly split but somewhat weak over the pulmonary region With a maximum parasternally on the left side in the third intercostal space a grade-4 pansystolic murmur was heard No diastolic murmur was auscultated

ECG showed atrial fibrillation and right bundle branch block Table I shows the results of the work tests, X ray photographs of the heart and heart catheterization

Angiography from the left ventricle did not show any septum defect. On the other hand it revealed the existence of a 3–4 mm wide communication between the right coronary sinus and the upper part of the outflow area of the right ventricle However it was not considered that an operation on this fistula was indicated as the patient had improved though to a minor extent The atrial fibrillation was regularized by DC shock to a permanent sinus rhythm

DISCUSSION

In 1936 Abbott (1) reported on 85 cases of Fallot anomaly The average length of life without surgical treatment was 12 years However in the literature several cases have been described in which the patients attained ages over 40 years Bowie (3) examined the literature and gave an account of 20 patients who had reached the age of 40 years or more The oldest was a 69 year-old man and Bowie herself described a 68 year-old woman Of the 20 patients only six were certainly cyanotic at birth four had been cyanotic as far back as they could remember one had noticed cyanosis at the age of 30 and in six cases cyanosis first began in middle life In two cases the age at which the cyanosis began was not stated and one patient had never been cyanotic

In patients with Fallots anomaly there is a positive correlation between the degree of cyanosis and the degree of pulmonary stenosis With increasing age progressive myocardial fibrosis and muscular hypertrophy arise in the infundibular type of pulmonary stenosis (4) The stenosis becomes more marked and explains the first appearance of cyanosis in middle life Our case 3 is of this type He had earlier been regarded as an acyanotic case of Fallots anomaly only in the last year had a right–left shunt arisen with cyanosis and a great deterioration in health That the degree of cyanosis is of great prognostic significance may be illustrated by the survival time in another cyanotic congenital defect viz. pulmonary atresia In these cases the stenosis is maximal and the patients seldom survive beyond childhood As far as we know the oldest patient described attained the age of 33 years (7)

Another important prerequisite for long life in Fallots anomaly is probably a well-developed collateral circulation from the bronchial vessels Probably the greater the bronchial circulation and the more blood handled by the left ventricle the better developed this ventricle becomes and the greater the systemic flow and oxygenation Burch et al (5) studied the electrocardiograms of 140 cases They found a well developed left ventricle in 11 cases The average age of these patients was 32 years and varied between 17 and 44 years The developed left ventricles of these patients may of course also have existed from birth and in themselves have been the prerequisite for long survival The best example of a well-developed collateral circulation with the bronchial circulation is a direct connection between the aorta or a systemic artery and the pulmonary artery Miller et al (10) also showed that in cases of Fallots anomaly which had previously been operated on with a shunt ad modum Blalock or Pott the left ventricular volumes had considerably increased if the left to right flow was large enough to cause symptoms On the other hand 12 out of 14 unoperated cases had volumes at the lower normal limit The two other cases were both cyanotic and both had significantly reduced enddiastolic volumes It is interesting to note that none of our three patients had electrocardiographic signs of left ventricular hypertrophy On the other hand left ventricular enlargement was demonstrated roentgenologically in the two oldest cases while the youngest man had signs of enlargement of both the right and the left ventricle However the X ray diagnosis of left ventricular enlargement is very uncertain if angiography is not carried out This particularly applies to the large heart

In cases of Fallots anomaly the second heart sound is often heard faintly and not split over the base depending on whether the pulmonary component is heard very faintly or not at all This is due partly to the reduced flow through the valve and partly to the cusps being thickened Another factor of importance may be dorsal displacement of the pulmonary valve Bousvaros (?) examined patients with Fallots anomaly usin intracardiac phonocardiograms and found that the pulmonary component was invariably recorded With this technique the distance between the pulmonary valve and the chest wall will not influence the detection of the second heart sound Of the 11 patients seen by Burch et al (5) who had an average age of 32 years and showed signs of well developed left ventricles 50% presented a distinct and split second sound over the base of the heart On the basis of these findings they speculated that the distinctly split second heart sound in these cases was due to the fact that the well-developed left ventricle prevented the pulmonary valve from being displaced too far dorsally In our three cases the second heart sound was weakened in the two oldest patients and the youngest man had a somewhat accentuated second sound over the base In no case was the sound split

Case 1 displayed a pronounced hypoxaemia with a high haematocrit value. This patient also had thrombocytopenia and a pathologically reduced thrombotest (42 %). It is well known that patients with cyanotic defects frequently display thrombocytopenia and also disturbance in the coagulation mechanism, possibly due to anoxic liver damage. Somerville (12) also found a deficiency of fibrinogen and a reduced production of thromboplastin. The greatest reductions were sustained by the patients with the highest haematocrit value. Kornhuber and Gutheil (9) among others have put forward the hypothesis that the thrombocytes are sensitive to anoxia and that their survival time is thereby reduced. It also turns out, as in our case, that after complete correction the thrombocyte count and the coagulation mechanism are normalized. However, in case 1 the haematocrit value remained increased postoperatively in spite of a normal arterial oxygen saturation. The explanation of the patient's high haematocrit value is probably that he has congenital cystic kidneys with a reduced renal function.

Cooley et al. (6) reported the results of complete correction in seven patients who were more than 35 years of age (the oldest was 45). Of these seven patients three were acyanotic and two died during the operation (both were cyanotic). Friesinger and Bahnson (8) described a 54-year-old man operated on with complete correction. A systolic pressure gradient of 100 mm Hg was recorded over the pulmonary ostium. The authors point out that the stenosis had probably progressed during the last few years, as the patient had previously been relatively free from symptoms. There was considerable stenosis also in our three cases. In the two oldest it is probable that earlier the stenosis was slight. The oldest man had performed relatively heavy manual labour. During the last year before the operation a great deterioration in health took place with increasing cyanosis. In this case there was only a moderate improvement postoperatively, as in case 2. However, it must be emphasized that these relatively elderly patients probably have a reduced myocardial function due to fibrosis. Case 2 also had chronic bronchitis and case 3 had postoperative atrial fibrillation, which was regularized to permanent sinus rhythm. The fistula from the right coronary sinus of the aorta to the outflow area of the right ventricle, which was probably due to surgical trauma, has not yet been dealt with. The risk involved in a second surgical intervention was adjudged to be great in this patient, who is now more than 60 years old.

Case of Fallot's anomaly have a reduced pulmonary blood flow and often a high blood viscosity. This probably explains the high frequency of thromboses in the pulmonary blood vessels of these patients. It seems possible that these patients may be more susceptible to respiratory tract infections with symptoms such as those of chronic bronchitis, as in our case 2.

We consider that surgical treatment should be offered in all cases of Fallot's anomaly. In patients less than five years of age a palliative shunt operation should first be performed. Complete correction may conveniently be carried out at the age of about 12. Complete correction is also recommended in middle aged patients, even though it seems probable that these patients will run a greater surgical risk on acount of impaired myocardial function. These patients also have a greater likelihood than children of having other diseases of a non-cardiac nature. Complete correction is preferred to a shunt operation in middle aged patients, as these patients have already shown by their long survival time that they have a good collateral circulation with the bronchial circulation. On account of the myocardial fibrosis and general tissue degeneration in middle aged patients, the post-operative improvement will be less striking than in children.

CONCLUSION

The three most elderly cases in a series of 56 consecutive operations for the complete correction of Fallot's anomaly are presented. At the time of the operation the three men were 43, 55 and 56 years of age. All three were studied by pre- and post-operative heart catheterization and angiography. In all three a systolic pressure gradient (RV-PA) exceeding 100 mm Hg was noted. All had both valvular and infundibular pulmonary stenosis. The youngest patient had only a right-left shunt at rest, while the two others had a bidirectional shunt. All three survived the operation. In the youngest a very satisfactory improvement was noted, while the improvement in the two oldest cases was only

moderate Post-operatively the oldest man had a fistula between the aorta and the outflow area of the right ventricle which probably occurred during the operation

The factors of importance for long survival in cases of Fallot s anomaly are a slight degree of pulmonary stenosis and a good collateral circulation with the bronchial circulation Probably the surgical risk is greater in middle aged patients than in children Less post-operative improvement may also be expected However surgical treatment of Fallot s anomaly should be offered in all cases

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DETERMINATION OF PHYSICAL WORK CAPACITY AND EXERCISE TOLERANCE IN CARDIAC PATIENTS

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Abstract Twenty four coronary heart patients and eight cardiac neurotics were tested physically by work loads up to maximum in order to assess their aerobic power and evaluate their work tolerance The average aerobic capacity in both groups was reduced by 30% The highest mean heart rate and the maximal oxygen pulse were also reduced. The average heart volume was normal In coronary heart patients the systolic B.P during exercise at high work loads tended to be low and ECG changes occurred in two thirds of them The cardiac neurotics did not show these cardiac abnormalities Only few of the coronary heart patients were able to perform heavy muscular exercise completely without signs and symptoms indicative of impaired myocardial function

The functional evaluation of cardiac patients with manifest decompensation usually involves little difficulty because the symptoms at rest provide sufficient guidance It is much more difficult to assess the functional capacity for work and exercise tolerance in patients who are well compensated at rest

The clinical findings do not afford quantitative measurements of the functional impairment and the history may often be misleading because the patients tend to exaggerate certain symptoms and hide others Therefore a quantitative measurement of the patients ability to perform physical work would be of great value on the one hand to prevent overestimation of the function on the other and perhaps more important to prevent underestimation

For the practice of medicine such objective measurements would be invaluable for the follow up of cardiac patients and would also be an important aid in the rehabilitation

An evaluation of work capacity and exercise tolerance in man may be based upon measurements of maximal power output and capacity of the bioenergetic mechanisms Since the oxidative process is the reaction which provides the major part of the energy to all daily life activities the measurement of the maximal power of the aerobic energy yielding process is a most important parameter The maximal power of this mechanism is determined by the measurement of maximal oxygen uptake

The maximal oxygen uptake depends on the type of exercise performed and the mass of muscles employed in the activity The highest values are obtained in such activities as running bicycling etc Large enough muscle groups are brought into play during these activities to load the oxygen transporting system to or close to the maximum of its capacity The critical size of the muscle mass which has to be utilized in order to bring about the highest maximal oxygen uptake has not been specified Maximal arm work yields a lower oxygen uptake than does leg work (9) There are small differences in maximal oxygen uptake when tested in such types of activities as running stepping and bicycling (9) but if test procedures are carefully standardized the reliability is good

The true maximal oxygen uptake depends on the functional dimensions and capacities of the oxygen transporting system All available evidence indicates that the maximal oxygen uptake is limited by the functional capacity of cardiovascular performances and the former therefore provides a measure of the latter

The criterion used to establish maximal oxygen uptake is to test the subject at gradational increases of work load finding the load of work at which a further increase in work output does not bring about any higher oxygen uptake The blood lactate level at the end of exercise may provide a supplementary criterion as a high blood lactate

indicates utilization of the maximal oxygen uptake power

In cardiac patients the maximal work which can be performed may be limited by symptoms and signs from the cardiac muscle When testing diseased subjects it is consequently necessary to stop the work before the criterion of maximal oxygen uptake is fulfilled and the level of work where signs and symptoms occur may be defined as the maximal work rate In coronary patients it is possible to define one or more of the following criteria for maximal work

1 typical anginal pain
2 occurrence of pathological ECG pattern indicating myocardial ischaemia
3 excessive dyspnoea
4 le elling off of oxygen uptake the normal physiological response

The occurrence of angina pectoris ECG changes or dyspnoea gave no indication for stopping the testing procedure during this investigation In all cases the testing session was fulfilled

The purpose of this study is to present data of maximal oxygen uptake and related respiratory and circulatory data in a group of postcardiac infarct patients and in a group with neurotic heart disease

MATERIAL

Thirty three subjects suffering from cardiac disease were examined They represented a special category of patients as most of them were recruited from rehabilitation institutions Twenty five of them suffered from coronary heart disease (? heart infarction and three typical angina pectoris) and the rest eight subjects from cardiac neurosis The time elapsed since the patients were admitted to hospital averaged -0 months Only two patients including one neurotic were partly at work The others had not gone back to their usual activities Some physical characteristics of the subjects are given in Table I and their different occupations in Table II

In eight of the subjects a thorough clinical examination including history did not reveal any organic heart disease nor did the information collected from hospitals and physicians Consequently they were classified as having functional cardiac disease i.e cardiac neurotics

One of the coronary heart patients was excluded from the material He was out for psychical reasons unable to collaborate in the testing procedure Of the remaining -1 subjects suffering from heart infarction ten had an anterior infarction nine a posterior and two both an anterior and a posterior infarction The dimension of the infarction process and the severity of the disease were most variable All of the examined subjects were in sinus rhythm Five of the coronary heart patients had a resting diastolic B P sitting on the bicycle in the range 100–110 mm Hg In one of the neurotics 100 mm Hg was noted

Table I *Physical characteristics of cardiac patients*

Mean ± S.D and range

Patients	No	Age (years)	Height (cm)	Weight (kg)
Coronary heart disease	24	50 ± 6 0 (35 – 59)	173 ± 5 8 (163 – 185)	74 0 ± 7 6 (6 5 – 94 3)
Cardiac neurosis	8	49 ± 6 8 (38 – 57)	175 ± 7 9 (163 – 187)	75 2 ± 8 5 (61 0 – 86 0)
Total	32	50 ± 6 1 (35 – 59)	174 ± 6 3 (163 – 187)	74 3 ± 7 7 (61 0 94 3)

Table II *Occupational categories of 32 cardiac patients*

	Cardiac disease	
Occupation	Coronary	Functional
Manual work		
Industry	3	?
Forestry/agriculture	6	2
Building/construction	7	3
Seafaring /fishing	2	1
Transport	4	0
Non manual work	2	0
Total no of pat	?4	8

METHODS

The clinical examination included ECG with 1 leads and X ray of heart and chest in the erect position The heart volume was determined according to Jonsell

Maximal oxygen uptake and related respiratory and circulatory functions were measured by having the subjects bicycle on an ergometer of the mechanical braking type On the first day of testing only a moderate work load was performed to accommodate the subjects to the work situation On the following days two load either two submaximal lasting 5–7 min or one submaximal and one maximal lasting 3–4 min were performed daily until the functional capacity was assessed The measurements were taken in the last minutes of the exercise periods

Respiratory measurements were taken by using an open circuit system expired air was collected into a balanced 150 l tank and samples of gas withdrawn for analysis by means of the ml Scholander method Ventilation volume and respiratory rate were simultaneously recorded on a kymograph

ECG at rest was recorded with the subjects in the sitting position on the bicycle Four CH leads cor

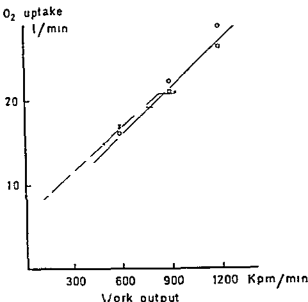


Fig 1 Oxygen uptake/work output relationship in 24 coronary heart patients, a era ed 50 years compared with old men reported by Be nestad (3) and young trained and untrained men reported by Hermansen and Anders_n (6) • coronary heart patients × old men 70–80 y □ male students 20–30 y O male athletes

responding to V V, V, V were used During the exer cise period the same four CH lead were regularly re corded at short intervals and compared to the resting ECG After the exercise the same procedure was repeated At maximal work loads an irregular base line and other disturbances could make the interpretation of the ECG difficult In the anal s s therefore a ST-depression of at least 1 / mm with a horizontal or downward sloping se ment was considered abnormal The heart rate was taken from the ECG in the last minute of the working period

BP was measured before and during exercise by the cuff method and w th a microphone placed over the bra hial artery

RESULTS

Oxygen uptake at submaximal work

The oxygen cost of bicycling at submaximal work rates is pre ented in Fig 1 The mean values for the cardiac patients are slightly higher than for young male students especially at low rates of work (6)

Maximal oxygen uptake

Table III lists the values for the highest meas ured oxygen uptake at work The coronary pa tients average 1 82 l/min or 25 ml/min/kg body weight The values for the neurotic patients are closely similar All the values are below the aver age 2 75 l/min or 37 ml/min/kg for the normal healthy population by about 30% (Fig 2) It is noted that the level of maximal oxygen uptake is

Table III *Maximal oxygen uptake and pulmonary ventilation*

Mean±S D and range

Patients	Max O_2 uptake (l min)	Max O_2 uptake (ml min kg)	Max. O_2 pulse	Highest pulm vent. (l min BTPS)
Coronary heart disease	1 82±0 35 (1 0?–? 40)	?5±5 (15–34)	12±1 7 (8–15)	57±16 (?9–93)
Cardiac neurosis	1 88±0 48 (1 33–2.79)	2?±6 (0–36)	13±?4 (10–17)	55±13 (4 –75)
Total	1 83±0 38 (1 0? 2 79)	25±5 (15–36)	12±1 9 (8–17)	57±15 (29–93)

approximately the same in those subjects who fulfilled the criterion of levelling off and tho e who did not

Heart rate

Heart rate is linearly related to oxygen uptake in both coronary and neurotic heart diseases and the average values for the two groups are iden tical The chara teristic is approximately the same as in healthy subjects though the slope of the regressional line tends to be somewhat steeper in cardiac patients than in normals The level of heart rate at for instance one l oxygen uptake is not much different (Fig 3)

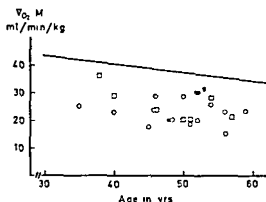


Fig 2 Maximal O uptake ml/min/kg body weight in relation to age The regression line for normal persons is drawn according to Hermansen (5) Open circles represent coronary heart patients and filled circles coronary heart patients with levelling-off of oxygen uptake Open squares represent cardiac neurotics and filled squares cardiac neurotics with levelling-off of oxygen uptake

Table IV *Heart volume and heart rate*

Mean±S D and range

Patients	Heart volume (ml)	(ml m²)	Highest recorded heart rate
Coronary heart disease	800±148 (500–1160)	4 0±79 (275–580)	151±19 (110–186)
Cardiac neurosis	700±139 (450–920)	380±68 (270–485)	149±19 (113–176)
Total	770±149 (450–1160)	410±77 (270–580)	150±18 (110–186)

The highest recorded heart rates maximal" heart rate are listed in Table IV and average 150 beats/min *in both categories of patients* This value is considerably lower than found in healthy men (Fig 4) It is clearly seen that the highest recorded heart rate is higher and closely similar to normal values in those subjects who performed work rates sufficient to bring about a levelling-off of the maximal oxygen uptake However in the other subjects the highest recorded heart rate is definitely lower in both categories of patients

Maximal oxygen pulse

The highest oxygen pulse is given in Table III and averages 12 ml in both groups of subjects There is no difference between coronary and neurotic cardiac patients In healthy Norwegian *men of the same age the average value is 15–16* ml (2 5) (Fig 5)

Arterial blood pressure

At rest in the upright position sitting on the bi cycle the arterial blood pressure was closely similar in the two groups of cardiac patients

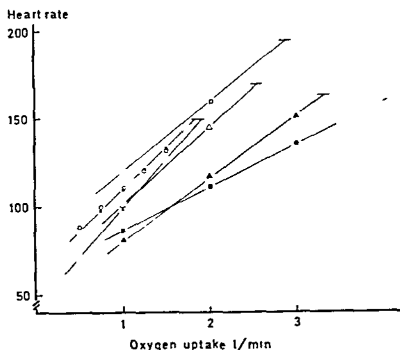


Fig 3 Heart rate/oxygen uptake relationship in coronary heart patients and cardiac neurotics compared with old men (3) middle aged trained and untrained men (?) young untrained men (1) and athletes (6) ● coronary heart patients O cardiac neurotics × old men, 70 83 y △ untrained men 50–60 y ▲ well trained men 50–60 y., □ young untrained men 20 y., ■ athletes 0–30 y

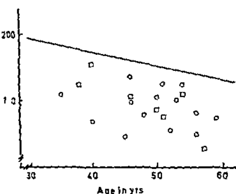


Fig 4 Maximal heart rate in relation to age The line presents normal values Symbols as in Fig 2

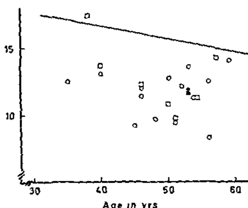


Fig 5 Maximal oxygen pulse in relation to age and compared with a regression line for normals taken from Andersen and Hermansen (2 5) Symbols as in Fig

averaging 133/93 mm Hg for the coronary subjects and 134/83 mm Hg for the neurotic cases In a normal group of trained males aged 40 years B P in the same position was lower and averaged 115/77 mm Hg (4)

Systolic B P increased in all subjects during work and for the neurotics in close linear relationship to the rate of work reaching values above 200 mm Hg in some of the subjects during maximal work and with average value 195 mm Hg For the coronary cardiacs the relationship was identical at light work rates but the average pressure curve levelled-off approaching maximal rates None of these subjects had a systolic pressure above 200 mm Hg and the average value during strenuous work was 180 mm Hg At, for instance a work rate of 500 kpm/min the systolic pressure difference in the two categories is statistically significant at the one per cent level As compared to normals B P values in both groups are higher at the same work rate (Fig 6) However in relation to the percentage of the highest oxygen uptake the B P response is approximately similar but tends to be somewhat lower for coronary patients at higher work loads

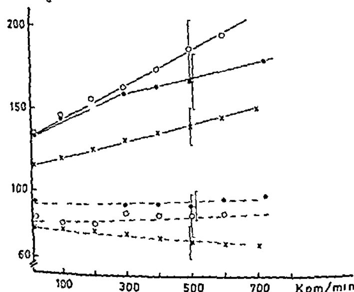


Fig 6 Blood pressure response to increasing work rates in coronary heart patients cardiac neurotics and a group of trained men, averaging 40 years (4) Vertical lines denote ± s.d ● coronary heart patients O cardiac neurotics × males averaged 40 y

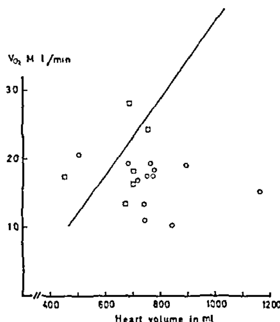


Fig 7 Heart volume related to maximal oxygen uptake The line is a normal one drawn according to calculations made from a diagram by Kjellberg et al (7) Symbols as in Fig 2

Diastolic pressure during work is difficult to measure accurately by means of the indirect un bloody method but the values indicate a small increase and slightly higher for the coronary pa tients

The pulse pressure in these coronary cardiacs will be lower with increasing work loads com pared to the neurotics and the normals

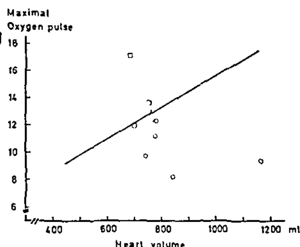


Fig 8 Relationship between heart volume and maximal oxygen pulse in cardiac patients The regression line for healthy people is given by Reindell et al (8) Symbols as in Fig 2

Volume of the heart

The volume of the heart averaged 800 ml for the coronary subjects and 700 ml for the neurotics (Table IV) Due to the large variation in this parameter the difference must be considered as insignificant and without any physiological im portance

The relationship between the volume of the heart and the maximal oxygen uptake is poor (Fig 7) It appears that most values obtained on cardiac patients are below the normal regression line (7) which means that for a given heart size the maximal oxygen uptake is lower than normal

The relationship between volume of the heart and the maximal oxygen pulse is also poor (Fig 8) Compared to the normal regression line for healthy subjects 40–59 years of age given by Reindell et al (8) it appears that the present data are distributed on both sides of the line but with the majority of values below the line According to the higher normal maximal oxygen pulse values in Norwegians this trend will in reality be more pronounced

Electrocardiogram

In the neurotic subjects no convincingly abnormal ECG changes were found For the coronary heart patients the changes during and/or after exercise are presented in Table V One patient is registered in two groups as ST-depression during exercise and ST elevation afterwards The same ECG also showed a sinus arrhythmia Another patient de veloped during work a ventricular conduction

Table V *Angina pectoris and ECG changes during and/or after exercise as compared to the resting ECG in 24 coronary heart patients*

ECG changes	Heart infarction	Angina pectoris
ST-depression	6	-
T wave inversion	1	
Both ST-depression and inverted T wave	3	
ST-elevation	3	
Both ST-elevation and T wave changed to positive	2	
No changes from rest	7	1
Anginal pain	8	2

One patient is registered in two groups as ST-depression d ing work changed to ST-elevation after work

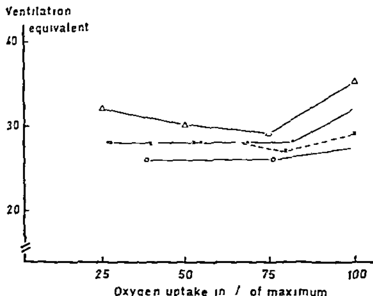


Fig 9 Ventilation equivalent related to the percentage of maximal oxygen uptake in cardiac patients compared to old men, reported by Benestad (3) and middle ag d men reported bv Andersen and Hermansen (2) △ old, untrained men, 70–83 y., ● coronary heart patients × cardiac neurotics ○ office workers and industrial workers 50–59 y

disorder with broader QRS-complexes Single ventricular ectopic beats were noted in a few subjects either at rest or during and after work load

The tim at which ECG changes related to "maximal" oxygen uptake occurred varied among the subjects from 44 % to 100% of the maximal uptake with a mean value of 75%. This phenomenon appeared independent of the limiting criterion of the aerobic power

Pulmonary ventilation

Pulmonary ventilation increases during exercise in linear relationship to the increasing oxygen uptake up to 75–80 of the maximum as in normal individuals A further increase of the oxygen uptake brings about a hyperventilation. The highest pulmonary ventilation is in both categories of patients somewhat lower than in healthy subjects and averaged 57 l/min as compared to approximately 80 l/min in normals of the same age (2 5) The difference is in relatively good agreement with the decrease in maximal oxygen uptake

The pulmonary ventilation efficiency is for the cardia patients lower than in normals The deterioration is however not so distinct as in old people (3) (Fig 9)

DISCUSSION

Th present study provides experimental data to evaluate the quantitative reduction of work capacity and exercise tolerance of coronary heart patients The highest oxygen uptake was on average 30% lower than the average maximal oxygen uptake of normal healthy men of comparable age and body size (2 5) This was not an unexpected finding but it was somewhat puzzling that the level of fitness was the same in patients with myocardial lesion as in th f w patient who were classified as neurotic cardiac patients

One of the most striking features in the testing of the postinfarction patients was that many were able to perform heavy rhythmical muscular exercise Nine of the 24 coronary subje ts succeeded in carrying out exercises sufficiently heavy to establish a plateau with regard to oxygen uptake This finding indicates a coronary blood flow capacity sufficient to meet the myocardium nutritional demand even during maximal loading though six of them showed ECG changes Their maximal power output was however reduced to the same extent as for the other tested subjects This low aerobic work power may be related to the myocardial lesion which may have reduced the mass of contractile structures thus imposing a cardiac limitation on maximal work output It is also possible that 20 months of physical ina tivity associated with the disease have resulted in a lowered functional status. If this latter concept holds true an activation regimen with physical training may have a good effect

The ma ority of true coronary patients 15 out

of 24 subjects had to stop the exercise test before the usual criteria of maximal work load had been established because signs and symptoms developed indicative of insufficient coronary blood flow These patients myocardial blood flow capacity is too low to secure proper nutrition to the contractile structures when the skeletal muscles are maximally loaded in bicycling and their work capacity and exercise tolerance is definitely limited by the coronary circulation

An infarct of the myocardium results in a reduced mass of contractile structures which may have several functional consequences One would expect a reduction in the contractile power which the myocardium may exert, and which would manifest itself in a reduced pumping capacity and a reduced ability to produce adequate pressures in the arterial system In the integrated organism one might analyse these effects by investigating the haemodynamic response pattern during graded exercise loading up to maximum involving measurements of cardiac output and the pressures in systemic and pulmonary circulation and comparing diseased subjects with normals

In this study no measurement of cardiac output was performed and the available data to analyse the problem are limited to heart rate and systolic systemic blood pressure related to the rate of oxygen uptake However simple as they are the measurements may yield an insight into the problem allowing some tentative conclusions

It should be noted that most of the coronary patients were able to raise their heart rate and systemic systolic blood pressure during exercise in close linear relation to the metabolic rate during submaximal work loads and their responses were quite similar to those which characterize the normal healthy sedentary man Approaching maximal work output the heart rate increase was as in normal subjects approximately linear while the pulse pressure of the systemic circulation in relation to oxygen uptake tended to decrease This indicates that cardiac output response to graded exercise in these coronary subjects is normal during submaximal loads During maximal work rate however an impaired functioning is likely to be present in most of these coronary subjects This statement is supported also by the lowered maximal oxygen pulse

The tentative conclusion to be drawn from this finding is that the myocardial lesion has only to a slight extent reduced the mass of contractile structures and the myocardial functioning below the limit required for adequate output at oxygen uptake up to the maximal level But since the maximal oxygen uptake is low reduced by 30° compared to normal subjects maximal cardiac output is most likely lowered to at least the same extent. It is not possible to say whether this reduction is related to a smaller mass of functioning contractile myocardial structures or whether it is a result of a reduced coronary blood flow capacity The distinction between these two parameters is mainly of academic interest From a practical point of view it is of less importance because under both circumstances the maximal output will be limited by cardiac performance The maintenance of adequate pressure relations in the systemic arterial circulation indicates that in most cases there exists a sufficient contractile power in the remaining myocardium to secure an adequate pressure drive in the circulatory system

It was quite unexpected to find that the maximal oxygen uptake in neurotic cardiacs was as low as in true coronary patients Only one of them was able to perform such heavy exercise as was required to fulfil the criterion of maximal oxygen uptake However the reasons for stopping exercise testing in neurotic subjects were not the same as for coronary subjects The neurotic subjects simply gave up when the exercise approached the maximal rate and no signs and symptoms of coronary insufficiency were recorded Their unwillingness and poor motivation to force themselves to heavy exercise are probably an effect or a part of the syndrome of mental instability or feeblemindedness which has imposed upon them a neurotic heart disease Their low aerobic work power may be related to physical inactivity possibly in some cases superimposed on an inherited physical inferiority Their normal circulatory responses to exercise indicate that the cardia muscle is not affected by any pathological condition

ACKNOWLEDGEMENT

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HAEMODYNAMICS IN ACUTE PULMONARY OEDEMA IN CORONARY PATIENTS

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Abstract Studies have been made of the haemodynamics in seven coronary patients with acute pulmonary oedema without shock Five of the patients had acute myocardial infarction The first examination was made 3–9 hours after the appearance of pulmonary oedema During the examination evident signs of pulmonary congestion and dyspnoea could be demonstrated The examination was made after treatment with oxygen Cedilanide® aminophyllin and Pethidin® The second examination was made 8 13 days later after all signs of pulmonary congestion had subsided In the acute state tachycardia reduced stroke volume and increased systematic blood pressure at a normal cardiac output were demonstrated The difference between the two examinations was an increase of stroke volume a reduction of the heart rate and systemic blood pressure and an increase of the relation between stroke volume and central blood volume

Pulmonary oedema is a well known clinical condition which in most cases is caused by an increase of the capillary venous pressure in the lungs of patients with congestive failure In some cases the cause is increased capillary permeability due to intoxication and other conditions

Haemodynamic investigations in acute pulmonary oedema are mostly made in patients with mitral stenosis who have developed pulmonary oedema during diagnostic heart catheterization Increases in the pressure in pulmonary capillaries and in the pulmonary artery reductions of the cardiac output and stroke volume and reduced arterial and mixed venous oxygen saturations have been demonstrated (6 8 10)

In mitral stenosis there seems to be a critical pressure in the pulmonary capillaries which depends on the plasma protein concentration (11 20)

About half of all cases of pulmonary oedema are caused by coronary heart disease (21) Haemodynamic examinations have been made in patients with acute myocardial infarction both with and without symptoms of shock (1 14 16 18 26 28) However few patients with acute pulmonary oedema without shock have been examined This study comprises patients with pulmonary oedema due to coronary heart diseases without symptoms of shock The examinations comprise heart rate cardiac index stroke index total and central blood volume arterial and central venous pressure The patients were examined twice as soon as possible after the beginning of the pulmonary oedema and again after all symptoms had subsided

The prognosis in pulmonary oedema is seriou Treatment with oxygen inhalation a digitalis preparation and aminophyllin (intravenously) and analgetics (as a rule Pethidin®) was given immediately after admission

MATERIAL

The material comprises seven patients with coronary heart disease without shock and without any other heart disease In five patients acute coronary occlusion could be demonstrated Five patients developed pulmonary oedema in the daytime in three of them following mild physical exertion Two patients developed pulmonary oedema in bed during the night Two patients died after the first examination and are not included in this study

The first examination was made from 3–9 hours after the first sign of pulmonary oedema All the patients had typical clinical symptoms at the time of examination with dyspnoea and pulmonary congestion The treatment had been started from –6 hours before the examination and the condition of the patients was somewhat improved

The second examination was made from 8–13 days after the first All signs of pulmonary congestion had disappeared and the patients were feeling well

In patients no 1 2 3 4 7 sinus rhythm was found Nos 5 and 6 had auricular fibrillation which persisted during both examinations

Except for the treatment the conditions were the same during both examinations

Table I *Haemodynamics in seven patients with acute pulmonary oedema Observations in acute stage and differences at later stage when the oedema had disappeared*

		BSA	CI	HR	SI	CVP_m	BAP_m	SR	MCT	TBV	CBV	CBV/TBV	SV/CBV
Ac stage	♂ 67	2 02	3 10	82	37 8	5 5	112	17 0	23 4	3021	1208	40 0	0 031
Later	Mb cord cor		−0 63	−25	+5 5	−1 0	−24	−0 3	+15 3	−2 7	−420	−11 8	+0 0?4
Ac stage	♂ 70	2.00	2.89	90	32 1	1 0	123	21 1	26 5	2905	1276	43 9	0 0?5
Later	Infarct ac		−0 56	−34	+9 5	+3 0	−29	−1 8	−1 9	−264	−3 0	−7 7	+0 018
Ac. stage	♀ 72	1 73	3 09	102	30 0	8 5	150	26 4	18 1	2494	935	37 5	0 032
Later	Mb cord. cor		−0 01	−39	+18 6	−5 5	−46	−7 5	+0 3	+3	+9	+0 3	+0 019
Ac stage	♂ 68	1 85	3 01	69	43 6	4 5	145	25 2	21 2	2715	1064	39 2	0 041
Later	Infarct ac		+0 15	−4	−5 1	−3 0	−42	−7 8	+2 2	+56	+172	+5 4	−0 002
Ac stage	♀ 75	1 57	2 39	118	20 3	4 0	97	24 8	24 1	2229	961	43 1	0 0 1
Later	Infarct ac		−0 22	−61	+17 8	+0 5	+9	+5 0	+0 9	+287	−55	−7 1	+0 0?1
Ac stage	♀ 64	1 71	2.92	94	31 0	0 5	93	18 6	20 3	3081	986	3 0	0 032
Later	Infarct. ac		+0 31	−13	−0 8	+1 0	+7	−0 7	−7 1	−467	−186	1 4	+0 006
Ac stage	♂ 61	1 69	3 63	1?1	30 0	2 0	154	24 8	28 4	3635	1850	50 9	0 016
Later	Infarct. ac		−0 ?8	−45	−14 1	0 0	−47	−6 2	−2 0	−585	−377	−2 6	+0 014
Mean ac stage			3 00	97	32.2	3 7	125	22 5	23 1	2869	1183	40 9	0 0?8
Mean diff			−0 18	−28	+10 0	−0 7	−25	−2 8	+1 1	−171	−168	−3 6	+0 014
				↓	↑		'						↑

Arrows denote significant differences ($P < 0.05$) other differences are not significant
BSA body surface area, m
CI cardiac index l min m^2
HR heart rate
SI stroke index ml/beat m^2
CVP_m mean central venous pressure mm Hg.
BAP_m mean brachial arterial pressure mm Hg.
SR systemic resistance arbitrary units ($BAP_m - CVP_m$/cardiac output)
MCT mean circulation time in seconds.
TBV total blood volume ml.
CBV central blood volume ml
SV stroke volume ml/beat

METHODS

The patients were examined in a heart bed in sitting position and with lowered legs Pure oxygen was given at atmospheric pressure with a closed mask A polyethylene catheter was introduced percutaneously into the arteria brachialis and into the cephalic vein The catheter was introduced without X ray control until it was considered that it lay intrathoracally The pressure was recorded by means of an Elema Schonander Transducer and Amplifier and registered together with the heart rate on a direct writing recorder The reference point was the 2nd intercostal space at sternum. Cardiac output and blood volume examinations were made after a single injection of 20 mg Evans Blue through a catheter by a rapid flush in system

The dye dilution curves were recorded from the arteria brachialis with a Cambridge cuvette and dye dilution recorder with a constant suction rate of 15 ml per min All blood samples were taken from the arteria brachialis for calibration after recirculation

Blood samples for plasma volume determinations were taken after 30 45 and 60 min The dye concentrations were examined with an Unicam spectrophotometer at the wavelength of 620 $m\mu$ The haematocrits were determined by Wintrobe s method and corrected for body haematocrit and trapped plasma with a multiplation factor of 0 9 The plasma volume was calculated by semilogarithmic plotting (and extrapolating) to the injection point Hamilton s semilogarithmic replotting method and formula were used for calculation of cardiac output

$$\text{Total blood volume} = \frac{\text{Plasma volume} \times 100}{100 - \text{haematocrit}}$$

$$\text{Central blood volume} = \frac{\textit{mean circulation time} \quad \textit{cardiac output}}{60}$$

Examinations of blood volume at one hour s interval with two dye injections in ten normal individuals did not show any significant difference between the two readings The mean difference was −137 ml the error of the method ($\sqrt{d^2/2N}$) was 4 2 % of the mean value of the two examinations

The corresponding figure for two examinations of the cardiac index in 15 normal individuals was −0 04 the error of the method 5 7%

RESULTS

The results are shown in Table I The statistically significant changes between the two readings were a reduction of the arterial blood pressure and heart rate an increase of stroke volume and an increase of the ratio between stroke volume and central blood volume A slight and insignificant fall in the mean values of cardiac output central venous pressure systemic resistance and total and central blood volume was also found

DISCUSSION

A difference has been demonstrated in the haemodynamics in acute pulmonary oedema compared with those found after symptoms had subsided

The absolute figures however must be evaluated with due consideration to the special conditions It has been demonstrated that oxygen inhalation reduces cardiac output and heart rate in normal individuals (3 4 12 13 19 29) in patients with acute myocardial infarction without pulmonary oedema (27) and in non-coronary patients with congestive failure (2) The arterial blood pressure may show a moderate increase in healthy persons (3) as in coronary patients (27)

Our patients had received a digitalis preparation (Cedilanid®) intravenously aminophyllin and Pethidin® In healthy people digitalis preparations have little effect on the haemodynamics (22 23 24) but in patients with congestive failure they reduce the blood volume (5) and increase the cardiac output and stroke volume (7 17 25) Aminophyllin has produced an increase of the cardiac output in left ventricular failure (30) and the sedative effect of Pethidin and the improvement of the dyspnoea may influence the haemodynamics (9)

The cardiac output we have registered in acute pulmonary oedema must be considered as normal As the heart rate is high the stroke volume must consequently be subnormal

Luisada (15) describes two types of pulmonary oedema. In the first type which may be seen in minor coronary heart attacks he found an increase of cardiac output and of systemic and pulmonary arterial pressure In the other type which appears in patients with massive myocardial infarction he found reduced cardiac output normal or reduced systemic blood pressure and a moderate increase in pulmonary arterial pressure

The material is too small to be considered from this point of view In our patients the combination of normal cardiac output tachycardia and increased systemic blood pressure is found Patients with signs of shock with a low cardiac output and systemic blood pressure are not included

The differences between the two examinations are moderate the chief difference being an increase of stroke volume without significant change of cardiac output

We have not been able to register the haemodynamics at the very beginning of the pulmonary oedema It is possible that the time which elapsed between the start of the oedema and the examination as well as the treatment may have improved the condition

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CHROMOSOME STUDIES IN POTENTIALLY LEUKAEMIC MYELOID DISORDERS

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Abstract Fifteen patients suffering from various non leukaemic" myeloid disorders have been cytogenetically investigated Evidence is presented that chromosome abnormalities may be present in both the erythrocytic and granulocytic precursors of the bone marrow On the basis of a review of the literature it is suggested that myeloid disorders with abnormal stemlines in the bone marrow should be considered neoplastic conditions It is concluded that the abnormal chromosome complements may be present at a very early stage of the leukaemic process and that the emergence of the abnormal stem lines occurs prior to and is unrelated to the blastic transformation of the granulocytic precursors characteristic of myeloblastic leukaemia

Despite the past ten years extensive cytogenetic research in acute leukaemia it has not been resolved whether the chromosome abnormalities demonstrated in the neoplastic cells of leukaemic patients represent an essential stage of the pathogenesis of the leukaemic process In order to elucidate this problem much interest has centered on chromosome studies in patients suffering from various haematologic disorders which occasionally may terminate in leukaemia e g polycythaemia vera myelofibrosis idiopathic thrombocythaemia, aplastic anaemia agranulocytosis sideroachrestic anaemia and conditions such as refractory anaemia or pancytopenia in which no underlying causes can be demonstrated For convenience these conditions will be designated myeloid disorders in the sequel

The aim of the present paper is to present cytogenetic data from 15 patients with myeloid disorders and to review the pertinent literature

MATERIAL AND METHODS

The patients were unselected Seven were males and eight females Their ages ranged from 38 to 74 years Prior to the study four patients had been treated with ionizing radiation or cytotoxics The remaining patients were untreated or had received treatment with anabolic steroids or corticosteroids The series encompasses patients with polycythaemia vera myelofibrosis idiopathic thrombocythaemia aplastic anaemia and pancytopenia (Table I)

In all cases chromosome studies were performed on bone marrow material In one patient with an abnormal stemline in the bone marrow the chromosome complements of the peripheral blood cells were also studied

For cytogenetic studies the marrow aspirates were treated according to the method described by Tjio and Whang (21) without prior in vitro culture Blood cultures were set up both with and without phytohaemagglutinin added according to the method of Moorhead et al (10) The former were harvested after 72 hours the latter after 24 hours

In one patient with an abnormal stemline in the bone marrow differential counts of the marrow cells and the mitotic figures were performed on Giemsa-stained smears of the same aspirate which was used for cytogenetic studies Mitoses were classified as erythroid only if the mitotic cells corresponded in size and tinctorial characteristics to proerythroblasts basophilic or polychromatic erythroblasts All other mitoses were considered as non-erythroid

RESULTS

The chromosomal findings are depicted in Tables II–III In two of the 15 patients abnormal stem lines were present in the bone marrow Normal chromosome complements were found in the remaining 13 cases In patient A (Table II) about 80 and 95 of the metaphases in the bone marrow aspirates contained 47 chromosomes The hyperdiploidy was due to an extra chromosome in group C (Fig 1) In the peripheral blood cells cultured for 24 hours without phytohaemagglutinin the same hyperdiploid mode was found In the blood cultures with phytohaemagglutinin

Table I *Types of myeloid disorders studied*

Types of myeloid disorders	No of pat. studied
Polycythaemia vera	6
Myelofibrosis	3
Idiopathic thrombocythaemia	2
Aplastic anaemia	2
Pancytopenia	2

added two stemlines were present viz. a normal diploid and a hyperdiploid stemline containing an extra chromosome in group C In patient B (Table II) about 60% of the bone marrow metaphases contained 48 chromosomes Karyotypic analysis revealed two supernumerary chromosomes in group C (Fig 2) No blood cultures were set up from this patient

Table II *Chromosomal findings in two cases of myeloid disorders with abnormal stemlines in the bone marrow*

Pat	Age (y)	Sex	Date	Survival from diagnosis	Type of tissue examined	Total cells counted	Chromosome number										
							<40	40	41	42	43	44	45	46	47	48	>48
A	70	♂	23 12.65		Bone marrow	19						1		2	16		
			27 1 66		Bone marrow	50						1		2	47		
			2 2 66		Peripheral blood without pha[a]	39						1	2	1	35		
			2.2.66	1½ y	Peripheral blood with pha[a]	43	1			1	3	2	3	18	15		
B	7.	♀	8 8 66		Bone marrow	50								19	2	79	

[a] pha = phytohaemagglutinin

Table III *Chromosomal findings in the bone marrow cells of thirteen patients with myeloid disorders*

Pat. no	Age (y)	Sex	Type of myeloid disorder	Date	Survival from diagnosis (y)	Total cells counted	Chromosome number										
							40	40	41	47	43	44	45	46	47	48	>48
1	43	♂	Aplastic anaemia	6 8 65	5½	35			1	1		2	3	.8			
2	61	♀	Poly-y theamia vera	3 9 65	2	4.						2		40			
3	38	♂	Aplastic anaemia	5 2.66	5	50							.	48			
4	39	♂	Polycythaemia vera	79 3 66	5	50						2	1	46			1
5	73	♂	Polycythaemia vera	27 4 66	11	50							3	47			
6	61		Pancytopenia	9 5 66	1½	50						2	1	46	1		
7	43	♀	Pancytopenia	25 7 66	8	50	1			1			3	45			
8	61	♀	Polycythaemia vera	12 8 66	3	50						1	1	48			
9	57	♂	Polycythaemia vera	17 8 66	17½	50							1	49			
10	48	♀	Idiopathic thrombocytheamia	1 9 66	3	50						1	4	44	1		
11	50	♀	Polycythaemia vera	5 10 66	2	50	.						4	44			
12	57	♀	Idiopathic thrombocythaemia	12 10 66	10	50								50			
13	74	♂	Myelofibrosis	5 11 66	5/1	50	1					1	4	44			

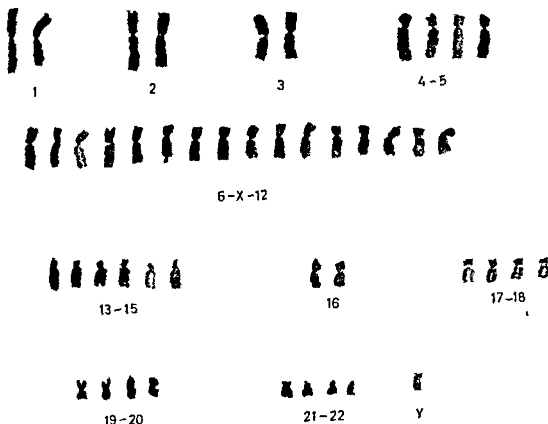


Fig 1 Marrow metaphase from patient A containing 47 chromosomes A supernumerary chromosome is present in group 6-X 1

As the course of disease in patient A gave rise to diagnostic difficulties a short description of the clinical and haematologic findings will be presented

The patient was a 70 year-old man In September 1965 he began to suffer from fatigue night sweats and abdominal distension During admissions to Medical Department B Bispebjerg Hospital and Medical Department A Rigshospitalet the spleen was found to reach the iliac crest and the liver five fingerbreadths below the costal margin In November 1965 the Hb concentration was 8 8 g ₀ and the leuko yte count 48 000/μl (4 ₀ myelocytes 6 metamyelocytes 20° juvenile leukocytes 52° polymorphonuclear leukocytes 16% lymphocytes and 2 ₀ erythroblasts) The red cells showed poikilocytosis and polychromasia Thrombocytes 40 000–70 000/μl The cellular compo ition of the bone marrow is depicted in Table IV A biopsy from the iliac crest showed sparse marrow elements Reticulum cells were the dominating cell type an increased number of megakaryocytes was also present and some collagen fibrils were seen From 25 11 65 to 5 1 66 650 r were given towards each of two tangential sections of the spleen resulting only in a slight splenic regression In January 1966 an increasing leukocytosis was seen (110 000–180 000 leukocytes/μl with 2° myeloblasts 4 promyelocytes 9 ₀ myelocytes 10 metamyelocytes 16 ₀ juvenile leukocytes 40 polymorphonuclear leukocytes 15% lymphocytes and 4 erythroblasts) The cellular composition of the bone marrow at this time is depicted in Table IV A biopsy from the liver showed cellular infiltrations in both the sinusoids and the portal spa es The patient then received treatment with busulphan 2–6 mg daily from 4 2 66–10 3 66 How

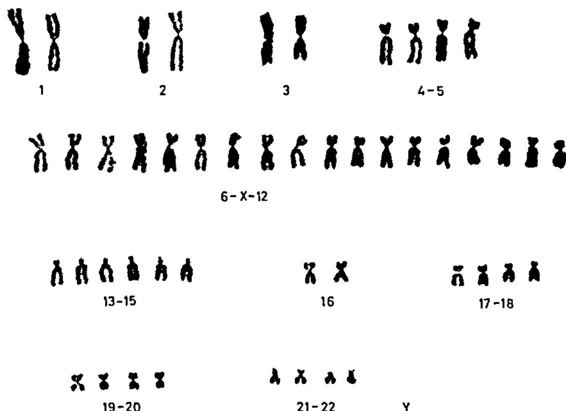


Fig 2 Marrow metaphase from patient B containing 48 chromosomes Two supernumerary chromosomes are present in group 6-X 1_

ever the condition rapidly deteriorated and death occurred at the end of March 1966 At autopsy the bone marrow was hyperplastic and dominated by immature myeloid cells The liver sinusoids red pulp of the spleen and lymph nodes were infiltrated with immature cells

The diagnostic possibilities in this patient were myelofibrosis and chronic myelocytic leukaemia The presence of numerous reticulum cells and megakaryocytes in the biopsy from the iliac crest and the initial leukocyte count of about 50 000/μl were in favour of myelofibrosis whereas the increasing leukocytosis (maximum 180 000/μl) and the decreased ratio of erythroid/myeloid cells in the bone marrow suggested chronic myelocytic leukaemia The presence of cell infiltrations in both the sinusoids and the portal spaces of the liver points both ways However the absence of

Table IV *Differential of cells and mitotic figures in bone marrow smears from patient A*

Date	Blast cells	Promyelocytes	Myelocytes	Metamyelocytes	Juvenile leukocytes	Polymorphonuclear leukocytes	Lymphocytes	Reticulum cells	Erythroblasts	Erythroid mitosis	Non erythroid mitosis
17 11 65	3	4	10	9	21	26	10	4	13	Not counted	Not counted
27 1 66	7	8	27	12	17	14	3	—	12	15	85

the Philadelphia chromosome in the marrow metaphases combined with some of the other features mentioned above make chronic myelocytic leukaemia less likely and seems to justify the classification of the disorder as an aggressive myelofibrosis with possible terminal transformation into acute leukaemia

Patient B was classified as myelofibrosis A biopsy from the iliac crest showed spongio a dominated by fibrillar connective tissue with sparse marrow elements Extramedullary haemopoiesis was found in biopsies from the liver and a lymph node One year after the chromosome study the condition has not shown any signs of transformation into acute leukaemia

All the patients with normal chromosome complements—except one—are still alive 5 to 66 months after the diagnosis One patient (no 13) died sudd nly five months after the chromosome study Autopsy was not performed None of the patients with normal chromosome complements in the bone marrow has developed acute leukaemia

From Table IV it is seen that 15 of the mitotic figures in the direct bone marrow smear from patient A belonged to the red cell series Thus the percentage of mitoses which occurred in the erythroblasts clearly exceeded the percentage of normal metaphases in the bone marrow aspirate which was used for cytogenetic study only 2 out of 50 metaphases being normal diploid

DISCUSSION

The present observations bear on two problems 1 the *distribution* of the chromosome abnormalities within the various cell types of the bone marrow and the peripheral blood from patients suffering from myeloid disorders and 2 the frequency with which abnormal chromosome complements are met in patients with ha matologic diseases which have some relation to leukaemia although they are ordinarily considered non leukaemi

Regarding the first problem the data obtained from the chromosome studies and the differential count of the bone marrow mitotic figures of patient A of the present series provides circumstantial—although indirect—evidence that at least in some patients with myeloid disorders the abnormal karyotypes are present both in the erythroid and the granulocytic precursors of the bone marrow Similar findings have been reported in acute leukaemia (7) and chronic myelocytic leukaemia (22 23 24) The event which gave rise to the alteration of the genome probably occurred in a stem cell common to the three cell types of the bone marrow The involvement of the two cell lines of the bone marrow—and possibly also the megakaryocytes—might explain why proliferative disturbances are often encountered in the granulocytic erythrocytic and megakaryocytic series in patients suffering from non leukaemic myeloid disorders

The results of the chromosome studies performed in cultured cells from the peripheral blood of patient A of the present series confirm the findings in previous series (8 12 15) Thus in blood cultures with phytohaemagglutinin added a majority of the metaphases had a normal diploid mode which indicates that the cells in the peripheral blood which are triggered into proliferation by phytohaemagglutinin—the lymphocytes—are not afflicted by the pathological process The abnormal metaphases demonstrated in these cultures and in the blood cultures without phytohaemagglutinin added probably originate from immature myeloid cells capable of dividing without stimulation by mitogenic factors

Regarding the second problem it has recently become apparent that abnormalities of the chromosome complement similar to those met in acute leukaemia may be present in the bone marrow and blood cells of patients suffering from non leukaemic myeloid disorders This applies to abnormal stemlines (9 12 14 15) structural aberrations (4 5) and an increased number of polyploid metaphases (15) Since abnormal stem lines are a characteristic of neoplasia this type of abnormal chromosome complement will be exclusively considered in the following review of cytogenetic studies performed in patients with myeloid disorders

In unselected series of patients with myeloid disorders abnormal stemlines have been encountered less frequently than in similar series of acute leukaemia Table V summarizes all available series of cytogenetic studies in patients with myeloid disorders A few patients have been omitted from some of the materials as leukaemia had probably developed at the time of the

Table V *The frequency of abnormal stemlines in the bone marrow aspirates of patients with myeloid disorders cytogenetically investigated*

Source of data	Cases examined (no)	Cases with abnormal stemlines (no)
Nowell & Hungerford 1962 (17)[a]	5	2
Sandberg et al 1964 (15)	20	1
Stafford et al 1965 (20)	18	2
Kay et al 1966 (4)[b]	40	13
Kiossoglou et al 1966 (5)	21	6[c]
Rowley et al 1966 (14)	15	3
Present series	15	2
Total	134	29

[a] *From this series two cases (nos 141 and 181) were omitted as leukaemia had probably developed at the time of the chromosome study*
[b] Three cases (nos 41 4? and 43) have been omitted from this series as leukaemia or a leukaemic state was present
[c] *In three of these patients chromosome studies were performed on peripheral blood cells only*

Table VI *The ploidy of abnormal stemlines in bone marrow material or peripheral blood cultures of 47 patients with myeloid disorders*

	No of pat
Hypodiploidy	8
Pseudodiploidy	16
Hyperdiploidy	23

Source of material
Sandberg et al 1960 (17) Nowell & Hungerford 1962 (1?) Solari et al 1962 (18) Freireich et al 1964 (1) Levan et al 1964 (9) Sandberg et al. 1964 (15) Speed & Lawler 1964 (19) Nowell 1965 (11) Stafford et al 1965 (20) de Grouchy et al. 1966 (?) Kay et al 1966 (4) Lawler et al 1966 (8) Rowley et al 1966 (14) Winkelstein et al 1966 (?5) Jackson & Huggins 1967 (3) Present material

chromosome study It is seen that abnormal stemlines were present in 22% of the patients In comparison in a recent compilation 122 of 246 patients with acute leukaemia were found to have had an abnormal stemline in the bone marrow (6)

The cytogenetic data available at present are too few and inconsistent to indicate whether abnormal clones are met more frequently in some types of myeloid disorders than in others Thus among 11 patients with polycythaemia vera who had not received treatment with ionizing radiation Kay et al (4) found five patients with aneuploid stemlines in the bone marrow whereas Kiossoglou et al (5) were not able to demonstrate abnormal chromosome complements in any of ten polycythaemic patients six of whom were treated with busulphan

In Table VI a review is given of the ploidy of the abnormal stemlines in 47 patients compiled from the literature It appears that hyperdiploid and pseudodiploid stemlines occur with highest frequency In contrast hypodiploidy and hyperdiploidy are about equally frequent in myeloblastic leukaemia with abnormal bone marrow stemlines (6) whereas pseudodiploid stemlines are infrequent (6) In this context it may be noted that the majority of patients with pseudodiploid stemlines in Table VI suffered from polycythaemia which had previously been treated with radio phosphorus Thus among 29 patients treated with P^{32} Kay et al (4) found eight patients with a pseudodiploid stemline in the bone marrow four of these were due to a deletion of a chromosome belonging to group F As already noted the same authors observed no pseudodiploid stemlines in eleven patients with untreated polycythaemia vera It is not possible to say whether these clonal changes are due to the treatment with P^{32} However Nowell et al (13) have recently demonstrated that ionizing radiation may give rise to pseudodiploid as well as aneuploid stemlines in murine bone marrow cells in vivo

When reviewing the karyotype analyses of the main aneuploid stemlines of the patients in Table VI it is found that in 25 out of 31 patients chromosomes belonging to group C were involved in the formation of the abnormal clones In 15 patients a clone with 47 chromosomes due to a supernumerary chromosome of group C was present In five patients an abnormal stemline with two supernumerary group C chromosomes was seen whereas a hypodiploid stemline due to a chromosome missing in group C was demonstrated in five patients The frequent involvement of chromosomes belonging to group C offers points of resemblance with the findings of acute leukaemia (16) In contrast the frequent involvement in acute leukaemia of group G chromosomes (16) has not been demonstrated in patients with non leukaemic myeloid disorders With the present cytologic techniques it is not possible to decide

whether the supernumerary or missing chromosomes of group C demonstrated in the aneuploid stemlines of these patients belong to the same or different pairs within the group

The significance of the frequent involvement of chromosomes belonging to group C is not known It has been described in patients with polycythaemia (4 9) myelofibrosis (3 5) idiopathic thrombocythaemia (14) aplastic anaemia (14) just as it has been demonstrated in cases which have terminated in acute leukaemia (1 15) as well as in cases in which no neoplastic transformation has occurred at the time of the report (5 14) One may speculate whether aneuploid cells produced by non-disjunction or partial endoreduplication involving a chromosome of group C may more readily acquire autonomous properties than other types of aneuploid cells and thus be able to give rise to abnormally proliferating clones

Recently some evidence has been presented which suggests that certain myeloid disorders may be associated with specific chromosomal changes Thus in five out of six patients with sideroachrestic anaemia de Grouchy et al (2) demonstrated nearly identical pseudodiploid stemlines which were due to an abnormality involving one of the chromosomes belonging to group F At present however too few patients have been cytogenetically investigated to decide whether this type of chromosome aberration is specific for sideroachrestic anaemia

Do cytogenetic investigations in patients with myeloid diseases give any information as regards the nature of these disorders and have they been able further to elucidate the role of chromosome abnormalities in the pathogenesis of acute leukaemia?

The presence of abnormal stemlines is a characteristic finding in the neoplastic tissue of many patients with leukaemia and solid neoplasms whereas aneuploidy is not encountered in patients suffering from non neoplastic diseases apart from the congenital chromosomal disorders in which the cytogenetically abnormal cells are present in the various tissues of the organism Usually the myeloid disorders are not considered neoplastic and the present review also demonstrates that abnormal clones are present much less frequently in these patients than in patients with acute leukaemia It must be taken into consideration however whether myeloid disorders with abnormal stemlines in the bone marrow should not be considered neoplastic conditions even though cytological examination of the bone marrow does not reveal any signs of neoplastic transformation A few recent findings suggest that this concept may be correct Freireich et al (1) studied three patients with refractory anaemia thrombocytopenia and granulocytic hyperplasia of the bone marrow The bone marrow cells of the three patients contained 45 chromosomes due to a missing chromosome of group C Two of these patients developed leukocytosis with immature leukocytes in the peripheral blood resembling chronic myelocytic leukaemia and terminated in acute myelomonocytic leukaemia Neither had a Ph[1] chromosome Recently Nowell (11) studied the chromosome patterns of 23 patients with myeloid disorders and found that subsequent transformation into acute leukaemia occurred more frequently in patients with abnormal clones in the bone marrow than in cytogenetically normal patients

On the other hand a few patients with myeloid disorders have survived for up to 3 1/ years with abnormal stemlines in the bone marrow without showing signs of a neoplastic transformation (11) Due to these findings it has been proposed (14) that abnormal stemlines per se are not indicative of neoplasia but that the abnormal clones may be more susceptible to the action of oncogenic agents than normal diploid cells It is equally justified however to consider abnormal stemlines in patients with protracted myeloid disorders as representing neoplastic clones which do not lead rapidly to a deleterious state either due to a low virulence" of the cell line or an increased resistance of the organism which may inhibit the proliferation of the abnormal clone That a long standing balance may exist between the host and what clearly is a leukaemic process is well known from the clinical observations of smouldering leukaemia

In conclusion cytogenetic studies in patients with myeloid disorders have not been able to define exactly the role of chromosome abnormalities in the pathogenesis of leukaemia They have demonstrated however that the abnormal chromosome complements may be present in all cell types of the bone marrow at a very early stage of the leukaemic process and that the emergence of the abnormal stemlines occurs prior to and is

unrelated to the blastic transformation of the granulocytic precursors characteristic of myeloblastic leukaemia

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THF EFFECT OF NEUROLEPTICS ON ELECTROCARDIOGRAMS

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Abs act The chan es observed in the ECG when chlor promazine and imipramine derivatives are given seem to be r-lated to the dosag used. The relati e Q-T duration however when chlorpromazine is administered in clinical dosage shows a highly significant prolongation When the effect of thioridazine and chlopentixol on ECGs was studied in forty patients the changes observed were sm l r b t s ghter and partly attributable to the tachy car a. There was a difference in the increase of the heart ra e in th groups of patients studied Nevertheless the incidence of T wa e depression was the same both in p tients treated with thioridazine in whom the increase in the heart rat during therapy was slight and in those treated with chlopentixol whose heart rate increased mo heavily An equal extension was noted in the rela tive Q-T dur tion In patients receiving neuro eptic drugs the magnitude of the ventricular gradient may be affected by the t chycardia The sh ft to the left observed in the direction of this grad ent does not, however allow any definite conclus on The result obtained suggests that the pathological component of th grad ent is identical with the T vector and the T-wave changes probably resulted from some pr mary chang in the repolarization due to the drugs tested

Ch'o promazine (6 13) and imipram ne deriva tives (10 14 15) are known to produce tachy cardia and in the electrocardiogram a depres sion of the T wave and condu tion disturbances On th other hand chlorpromazine doe not seem to produ e tachycardia or bundle branch blo k when given in clinical doses up to 6 mg per kg of body weight When the dosage is 150–300 mg da ly the relative Q T duration has however been found to be prolonged highly significantly (3) This has not been attributed to the increased heart rate produ ed by chlorpromazine at least not alone A delay in the duration of repolariza tion has also been noted by other authors (4 9) The present study is a comparison of the electro-cardiographic changes produced by other neuro-leptic drugs

MATERIAL AND METHODS

Thioridazine and ch openixol which are extensively used in psychiatrics, were se e ted as the drugs to be studied Thioridazine (Thioridazin hydrochloride Melleril San doz AG Basel) produces effects essentially similar to those of chlorpromazine although it seldom provokes extrapyramidal symptoms, and its side-effects and toxic manifestations are fewer (8) Furthermore it has only a weak anticholinergic action Chlopentixol (Sordinol Lund beck & Co., Copenhagen) is a tricycli psy hopharma ceutic with a carbon nu leu It is a st ong neuro epti of rapid action exerting a strong r adreno ytic eff ct than chlorpromazine It may also pro oke extrapyramidal symptoms su h as rigidity and tremo

The effect of these drugs on ECGs was stud ed by taking the ECGs 7 14 21 and, in some cases 28 days after the initiation of therapy and a week after its termination The ECGs were made and measured b the method previously descr'bed by u in a study on the e ectrocard ographic changes produced by chlorpromazine hydroch oride (3) Tachycardia was examined b com paring the heart rate in the ECG af er th first we k of therapy with that taken a week after ts comp etion Electrocard ographic changes noted in the course of therapy were compared with th ECG taken before its initiation In addition to the T wave and relati e Q-T duration the magn tude and direction of the ventricular grad ent were studied b planimetric measurement of the areas of QRS and T wa e in micro olt seconds (μVS) in the f ontal plane in the ST I and ST III limb le ds The area of QRS furthermore was measured separately using the formula the product of the base times height of the triangle when the pathological component of the ent icular gradient was stud ed The d re tion and the magnitude of the entricular gradient were obtained from a d agram presented by Lepeschkin (1) A planimeter was used (G Coradi Zur ch No 32761) The plani metric measurements were a r ed out by Ilkka Eskola M Sc

Forty patients were exam ned 23 of them were g ven th oridazine The dose given with two exceptions was 150 mg of thio dazine chloride per 4 hours in one case the dose was 2 5 mg and n the other 300 mg Chlopentixol was given to 17 patients 13 of them had a da ly dose of 60 mg, three had 75 mg, and one 150 mg

The age and sex of the pat ents examined are presented

Table I *The subjects studied by age distribution*

Drug	Age in years 15–19	20–29	30–39	40–49	50–53	Total no of cases
Thioridazine chloride	1 (0)	3 (1)	6 (3)	11 (4)	2 (1)	23 (9)
Chlopentixol	1 (0)	6 (3)	3 (3)	5 (?)	2 (1)	17 (9)
Total number of cases	2 (0)	9 (4)	9 (6)	16 (6)	4 (2)	40 (18)

The number of female subjects in brackets

in Table I The youngest was 15 and the oldest 53 years 36 were under 50 and four between 50–53 years All the patients were treated at the psychiatri clinic or out patient clinic of Helsinki University Central Hospital and the routine physical examination performed before therapy was initiated had revealed no changes in the circulatory organs

RESULTS

Sinus rhythm was present in all cases In 14 of the 23 patients treated with thioridazine the heart rate was found to have increased during therapy the range of the increase was 2–39% average 13% In two cases no change was noted and in seven cases the rate was reduced Of the 17 pa tients treated with chlopentixol the heart rate increased in 15 range 3–57 % average 23% In two cases it was reduced

T wave and relative Q T duration

The changes noted in T wave and relative Q T duration are shown in Table II In both groups the T wave was found to show a depression on an average of 18 and 19% respectively from the control value This change is almost significant The relative Q T duration showed an extension in both sections ranging from 2 to 5% The biggest change an extension of 5% was seen in the patients treated with thioridazine In both groups the change observed was almost signifi cant A study of the variation in T wave between the groups and the change in Q T duration re vealed no significant differences between the groups

Ventricular gradient

The minimum maximum and mean values for the magnitude of the ventricular gradient are

Table II *Changes observed in the T wave and relative Q–T duration following administration of neuroleptic drugs for seven days in two groups of subjects*

The drug used	No of cases (n)	T wave in millivolts: Control values Min	Max	Mean	Values after therapy Min	Max	Mean	Change ( )	t	P	Relative Q–T duration ( ): Control values Min	Max	Mean	Values after therapy Min	Max	Mean	Change ( )	t	P
Thioridazine chloride	23	−0 20	0 50	0 286	−0 10	0 50	0 235	−17 8	2 76	0 05	80	105	99 3[a]	95	120	104 3[a]	4 6	2 66	0 05
Chlopentixol	17	0 10	0 45	0 288	0 05	0 40	0 232[b]	−19 4	2 28	0 05	95	110	102 4	95	115	104 6[b]	2 2	2 64	0 05

[a] n = 22 [b] In one case the value obtained after 21 days treatment has been used

Table III The magnitude and direction of ventricular gradient before and after treatment with neuroleptic drugs for seven days each in two groups of subjects

		Magnitude μVS									Direction degrees								
	No of cases	Control value			Value after therapy						Control value			Value after therapy					
The drug used	(n)	Min	Max	Mean	Min	Max	Mean	Change ( )	t	P	Min	Max	Mean	Min	Max	Mean	Change ( )	t	P
Thioridazine chloride	3	5	92	53 4	5	109	57 9	8 3	1 05		−30	108	48 3	−90	98	36 6	−22 8	2 12	0 05
Chlopenthixol	17	18	82	51 6	18	80	46 5	−9 8	1 02		−13	78	46 5	−52	7	36 8	−21 5	1 29	

given in Table III which also shows the changes in the direction of the gradient in both groups of the studied patients After therapy the magnitude was found to have increased by an average of 8 3° in the first group and to have declined by 9 8° in the second group The changes noted were not statistically significant and their mutual differences were not tested The change a fall of 22 8 ° noted after therapy in the direction of the ventricular gradient of the patients who were given thioridazine is almost significant The relevant mean change recorded in the subjects treated with chlopenthixol a fall of 21 5° is not significant The difference between the groups tested by variance analysis is not significant

DISCUSSION AND CONCLUSION

There were approximately equal numbers of men and women in both series The dose given in all *cases exceeded that generally recommended in* other than psychiatric therapy (8) Hence the amounts of drugs given probably played no part in the differences noted between the groups in heart rates From the tachycardia observed it appeared that the anticholinergic action of chlopentixol exceeds that of thioridazine On the other hand the heart rate of the patients during the therapy in both groups was found to have increased by a lesser degree than in six patients treated similarly with chlorprothixen a neuroleptic drug with clinical action and adverse effect approaching those of chlorpromazine (11)

When 50 mg chlorpromazine was given three times a day for several weeks a significant depression of the T wave was only noted after the first week of therapy (3) Similarly a highly significant extension of the relative Q T duration was observed already after the first week of therapy For this reason the present study was only concerned with the electrocardiographic changes seen after the first week of therapy The T wave depression noted was heterogeneous though no significant difference was seen between the groups in this respect The T wave depression percentage recorded for patients treated in this study was lower than that noted in connection *with the use of chlorpromazine (3) The extension* of the relative Q T duration was slight but uniform It seems therefore that electrocardiographic changes accompanying the administration

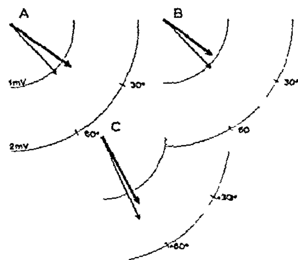


Fig 1 The orientation of the average ventricular gradient indicated by the arrow before (*thin arrow*) and after (*thick arrow*) treatment in subjects given thioridazine (*A*) and chlopentixol (*B*) For details see text

of the neuroleptics under study are slighter than those noted with chlorpromazine which seems readily to increase the relative Q T duration

The average values of the magnitude and the direction of the ventricular gradient are within normal limits (12) The changes observed are seen in Fig 1 The change found in the magnitude and direction of the ventricular gradient for the six patients treated with chlorprothixen a neuroleptic drug known to produce a marked tachycardia is given in the same figure The patients had a daily dose of 45 mg to 150 mg of chlorprothixen chloride given orally

In general the magnitude of the ventricular gradient varies too much from individual to individual under non pathological conditions to be of any value in a single electrocardiogram (7) In serial electrocardiograms also the change observed may be due to non pathological factors This was thought to be the case in probably all of the subjects studied As the duration of ventricular activity decreases with rising heart rate and vice versa and the ventricular gradient also parallels the reciprocal of the heart rate (2) the changes observed in the magnitude of the ventricular gradient are more likely to be due to the change observed in the heart rate and a direct effect of the studied drugs upon the myocardium may be of minor degree The heart rate was also found to bear a negative correlation to the absolute magnitude of the gradient in the subjects receiving chlopentixol showing a more pronounced tachycardia A definite conclusion could however not be made as the correlation was found not to be significant

The shift to the left observed in the direction of the ventricular gradient however slight was uniform and almost significant in the group of subjects treated with thioridazine This change even of moderate degree is more likely to be due to pathological changes (2) when it is observed without variations in the QRS complexes No significant change in the magnitude or direction of the area of QRS after treatment however was found when tested separately in the studied groups of subjects The pathological component of the ventricular gradient (12) was consequently thought to be identical with the pathological component of the T wave An almost significant lowering of the T waves was found in both groups studied This appears however to be not only due to tachycardia but possibly to some primary change in the repolarization resulting from the neuroleptic drugs in question

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ACID PHOSPHATASE ACTIVITY IN RHEUMATOID SYNOVIA

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Abstract The synovial membranes from two normal joints five joints affected by rheumatoid arthritis and two joints showing osteoarthritis were studied immediately after synovectomy Marked differences in acid phosphatase activity were noted the activity in rheumatoid synovia being twice as high as in the other two groups The granular fraction obtained by centrifugation showed a 28 fold increase in acid phosphatase activity as compared with the original whole homogenate of normal synovia In rheumatoid arthritis on the other hand there was an only twofold increase The significance of this finding from the standpoint of the rheumatoid tissue reaction is not clear but the results seem to show that the lysosomal membranes of the reumatoid tissue were fragile It should be borne in mind that agents which stabilize the lysosomal membrane have been shown to have a clinical effect on rheumatoid arthritis

In the synovial membranes of patients with rheumatoid arthritis a significant increase in acid phosphatase activity has been observed as compared with normal synovia (5) This finding was made in experiments performed on tissue homogenates No determinations of the enzyme activity in the different synovial subcellular fractions have been reported

Histochemical investigations have disclosed that the number of subcellular structures with acid phosphatase activity is larger in rheumatoid synovia than in normal synovia and the synovia from osteoarthritic joints (4) The activity of leucinoaminopeptidase an endopeptidase capable of hydrolysing peptides is increased in the synovia in rheumatoid arthritis (7)

The results of electron microscopic studies seem to corroborate the observations made with the aid of the light microscope Cytoplasmic granules with high acid phosphatase activity have been found in rheumatoid synovial membranes (1)

The free activity of autolytic enzymes is to be regarded as an active element in tissue destruction since it is not likely that the bound activity causes such a process In rheumatoid arthritis it is therefore important to study both the free enzyme activity in the synovia and the activity which is bound to the subcellular structures

The acid phosphatase positive granules have been regarded as lysosomes

MATERIAL AND METHODS

Synovial tissues were collected at operations performed at the Hospital of the Rheumatism Foundation Heinola Finland All synovectomies were carried out by the same method Particular care was taken to ensure that the material to be studied consisted only of synovial membranes Immediately after dissection the specimens were placed in ice-cold 0 25 *M* sucrose solution and frozen on solid carbon dioxide

A representative piece of each synovia was examined histologically A total of nine synovial membranes were investigated The distribution of the material was as follows two normal cases two cases of osteoarthritis and five cases of rheumatoid arthritis Of the last mentioned five patients two had received gold therapy immediately before operation

After slow warming the tissue was homogenized with Ultra Turrax tissue homogenizer at +4 C for four seconds Ultra Turrax was chosen because attempts at homogenizing the fibrous synovial tissue by other methods had failed Teflon pestle homogenizer Griffin & George Ltd 3000 rpm did not give satisfactory results Woessner (9) who worked with uterine tissue did not get satisfactory results either with the teflon pestle homogenizer Ultra Turrax is admittedly a far too destructive method of homogenization but it s the only efficient technique among those so far described Special care was therefore taken to standardize the size and shape of the specimen and the time of homogenization

The 1 10 synovia–sucrose homogenate was centrifuged by the method used for the collection of a so-called granular fraction of rat liver containing lysosomes (8) in

Table I *Synovial acid phosphatase activity expressed as liberated phosphate/min/g of protein in control and test groups*

Group	Case no	Age	Sex	Enzyme activity in the homogenate		Enzyme activity in the granular fraction	
				Free	Total	Free	Total
Controls	1	59	♂	15 6	24 1	646	676
	2	48	♂	*17 5*	*?0 6*	*500*	*640*
Mean values				16 5	22 3	500	640
Rheumatoid arthritis	1	22	♂	36 0	41 0	40 0	46 0
	2	61	♀	27 0	29 0	70 0	68 0
	3	62	♂	*29 0*	*38 1*	*78 8*	*1?0 4*
Mean values				30 7	36 0	62 9	78 1
Gold treated rheumatoid arthritis	1	50	♂	25 9	27 8	216	200
	2	37	♀	*25 6*	*22 0*	*78 2*	*78 2*
Mean values				25 8	24 9	147 1	139 1
Osteoarthritis	1	35	♀	14 3	15 5	39 9	52 3
	2	45	♂	*13 3*	*12 4*	*61.5*	*68 5*
Mean values				13 8	13 9	?0 7	60 4

a Spinco centrifuge at +4 C 800 × g for 10 min The supernatant was collected and centrifuged at 15 000 × g for ?0 min The last precipitate was regarded as the granular fraction

The acid phosphatase activity and the protein concentration were determined in the homogenate and in the granular fractions The total enzyme activity was determined in the presence of 0 1 per cent Triton X 100 in the incubation mixture Triton bursts the lysosome membranes

Acid phosphatase activity was determined with beta glycerophosphate as substrate at pH 5 (3) The incubation time was 15 min and the activity is expressed as liberated phosphate/min g of protein Protein determination wa carried out as suggested by Lowry

RESULTS

The results are shown in Table I The homogenized rheumatoid synovia exhibited the highest total activity of the enzyme the values being almost twice as high as those obtained on normal synovial tissue and on the synovia from osteoarthritic joints After Triton incubation the activity showed a greater rise in the normal synovia than in the other groups This seems to indicate that in rheumatoid arthritis the enzyme activity is not so firmly bound to the tissue structures as in normal joints Furthermore these results show that the lower total activity in osteoarthritis is not bound to the same degree as is the activity in normal synovia

The results on the granular fraction were consistent with those reported above When this fraction was separated by centrifugation the enzyme activity was recovered in the granules in the normal cases but in those showing rheumatoid or osteoarthritic disease it was lost in the supernatant This observation argues in favour of the view that in severe arthritic disease the enzyme activity is loosely bound and more easily mobilized for tissue destruction (Table II)

In one of the cases of rheumatoid arthritis treated with gold the values lay between those of normal and affected joints Whether this reflects a gold effect cannot be settled on the basis of the present results

Table II *Rise in en yme activity from homogenate to granular fraction*

Group	Rise
Control	2 850
Rheumatoid arthritis	117
Gold treated rheumatoid arthritis	457
Osteoarthritis	332

DISCUSSION

Our results according to which rheumatoid arthritis is associated with a high enzyme activity in rheumatoid synovia are in good agreement with those of Luscombe (5) It was not surprising to find that the focus of the destructive tissue processes exhibited an increased activity of autolytic enzymes The significance of this phenomen

is not quite clear but it has been supposed that the autolytic enzymes of the synovial pannus tissue are capable of destroying the cartilage (10) It is of course possible that the enzymes of cartilage tissue also exhibit this activity

Even considering that the method used for recovery of the granular fraction was crude the homogenization being destructive and centrifugation rou h the difference in activity between the granular fraction and the remainder is so marked that it cannot be disregarded The possibility of changes in subcellular particles and especially in the lysosomal membranes should therefore be taken into account in discussing rheumatoid tissue damage According to our observations it is obvious that these structures are impaired

Page Thomas (6) using the Gomori acid phosphatase method on unfixed cryostat sections (2) as an indicator of the lysosomal membrane permeability recorded a much more rapid definitely positive acid phosphatase reaction in rheumatoid synovia than in normal tissue This observation argues in favour of lysosomal membrane changes in rheumatoid arthritis and corroborates the present results Against this background the untoward effect of ultraviolet radiation streptolysin S and vitamin A on diseases of the connective tissue is understandable These factors affect the stability of the lysosomal membranes Correspondingly the effect of cortisone and chloroquine on rheumatoid inflammation may be attributed to their stabilizing influence on the lysosomal membranes

Gold therapy has many advocates Gold probably has an inhibiting effect on the autolytic enzymes Whether gold stabilizes the membranes is not known

It is futile to speculate as to what agents may be responsible for the deterioration of the lysosomal membranes in rheumatoid arthritis It is possible that the decreased pH resulting from increa ed lactic acid production accounts for the phenomenon as was suggested by Luscombe (5)

The low total activity in synovia showing arthrotic changes may perhaps be interpreted as a manifestation of reparative fibrosis Such a process ought to be reflected in a lowered activity per gram of protein The observation that membrane fragility was the same in the granular fraction of the synovia in osteoarthritis and rheumatoid arthritis la ks an explanation

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THE EFFECT OF SHORT TERM EXERCISE ON PLASMA VOLUME AND BLOOD PRESSURE IN GUANETHIDINE TREATED HYPERTENSIVES

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Abstract Short term exercise produced a decrease in plasma volume and a rise in B.P in normotensives and untreated hypertensive patients The decrease in plasma volume is presumably due to an increased intra-capillary pressure in the contracting muscles In guanethidine treated patients there was no change in plasma volume during exercise probably because of vasodilatation and decreased intra-capillary pressure in the contracting muscles In several of the guanethidine treated patients B P fell during and after exercise in some cases so markedly that symptoms of cerebral ischemia developed

It is generally accepted that plasma volume decreases with short term exercise in both normal individuals (2 5 10 14 18) and in patients with congestive heart failure (8 10) Several studies have shown that ganglionic blocking agents such as hexamethonium and pentolinium (7 15) as well as sympatholytic agents such as bretylium and guanethidine (3 4 9 19) are able both durin and after exercise to prevent an increase in blood pressure or even produce so pronounced a fall that syncope or dizziness results (3 4 9 16 19) Previous studies have shown that the hypertensive action of the above mentioned agents decreases with time because of either fluid retention or increasing blood volume (12 17) The present study was conducted in order to determine whether changes in plasma volume accompany the decreases in B P seen in association with exercise in guanethidine treated patients For comparison the effects of short term exercise on B P and plasma volume in patients with normal B P and with untreated hypertension were also studied

MATERIAL AND METHODS

Three groups were studied nine men and one woman with normal B P six men with untreated hypertension and finally six men and five women with hypertension treated for several years with guanethidine (12 to 50 mg a day) plus hydrochlorothiazide with KCl (25 to 100 mg a day) under regular control in this department None of the individuals studied had any signs of congestive heart failure All studies were conducted after the subjects had been both resting in the supine position and fasting for at least three hours Short term exercise was accomplished in the sitting position on a stationary bicycle ergometer the patients presenting on the average 400 kg m/min for ten minutes B P was measured in the sitting position and is given as the average of 3 to 4 measurements which were taken during three periods the 15 minutes just before exercise the 10 minutes of exercise and finally the first 10 to 15 minutes after exercise Plasma volume was determined using ^{131}I albumin according to a previously described technique (6) only one blood sample for counting the degree of dilution after injection of ^{131}I albumin being taken Thus no correction was made for extravascular escape of ^{131}I albumin For determining plasma volume before exercise 5 μC ^{131}I albumin were given Twenty minutes later a blood sample was taken for the determination of plasma volume hematocrit and serum sodium Following this 30 μC ^{131}I albumin were given and immediately after exercise a new blood sample was taken and plasma volume hematocrit and serum sodium were determined Plasma volume was calculated as the ratio between total injected activity and the activity increase per ml plasma 20 minutes after the injection of ^{131}I albumin

RESULTS

Normotensive patients

The effect of short term exercise on B P plasma volume serum sodium and hematocrit is shown in Table I Mean B P increased during exercise in all patients and the average of 20 mm Hg is significant (S E M = mean error = ± 3 mm Hg $p < 0\,001$) After exercise the B P returned to the pre exercise level Plasma volume decreased between 2 and 10 with a mean value of 6 % (164 ml) which also is significant (S E M = ± 29 ml $p < 0\,001$) The decrease in plasma volume

Table I *Effect of exercise in normotensive patients*

			Blood pressure (Mean) (mm Hg)			Plasma volume (ml)			Serum sodium (mEq/l)		Hematocrit ( )	
Pat. no	Sex	Age	Sitting, rest	Sitting exercise	Sitting post-exercise	Before exerc	After exerc	Per cent change	Before exerc	After exerc	Before exerc	After exerc
1	♂	40	113/70 (92)	137/53 (95)	107/70 (89)	2817	2673	(−5 0)	146 5	145 0	46 0	47 0
2	♂	43	125/80 (103)	161/84 (123)	124/83 (104)	3164	3005	(−5 1)	—	—	41 5	—
3	♂	38	130/80 (105)	163/78 (12)	127/80 (104)	2811	2746	(−2 3)	144 0	142 0	48 5	49 0
4	♀	21	132/80 (106)	177/73 (125)	123/80 (102)	2596	2371	(−8 7)	140 0	141 5	48 5	51 0
5	♀	40	122/82 (10)	160 87 (124)	155/82 (99)	2999	2753	(−8 2)	144 0	144 0	46 0	47 0
6	♀	42	137/88 (113)	172/107 (140)	135/93 (114)	2096	2021	(−3 6)	144 5	144 0	45 0	46 0
7	♂	64	132 88 (110)	188/93 (142)	145/100 (123)	3041	2846	(−6 4)	144 0	143 5	43 5	45 0
8	♀	41	113/70 (92)	138/70 (104)	115/72 (94)	2786	2573	(−7 7)	—	—	40 5	41 0
9	♂	62	135/90 (113)	173/113 (143)	133/90 (112)	2439	2391	(−2 0)	146 0	147 0	39 0	39 0
10	♀	57	107/73 (90)	137/82 (110)	113/82 (98)	2567	2299	(−10 4)	14 0	143 0	42 5	44 0
Mean			125/80 (103)	161/84 (123)	124 83 (104)	2732	2568	(−6 0)	143 9	143 8	44 1	45 4
Change ±S E.M			+20±3 ($p<0\,001$)			164±29 ($p<0\,001$)						

[a] Mean B P = (systolic + diastolic)/2

was accompanied by an increase in hematocrit while serum sodium remained unchanged Changes in mean B P and plasma volume in association with exercise are given in Fig 1 As can be seen exercise produced an increase in B P and a decrease in plasma volume in all patients

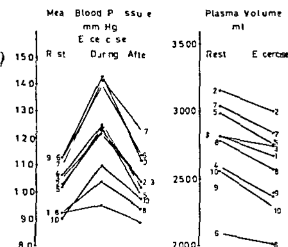


Fig 1 The effect of short term exercise on mean blood pressure (systolic + diastolic) and plasma volume in ten normotensive patients The number beside each dot indicates patient no

Untreated hypertensive patients

Changes in B P and plasma volume show the same pattern as in the normotensive patients (Table II and Fig 2) There was a significant rise of 22 mm Hg in mean B P (S E M = ±7 mm Hg < 0 025) and a significant decrease of 10° (260 ml) in plasma volume (S E M = ±58 ml $p<0\,01$) which was accompanied by an increase in hematocrit with no change in serum sodium B P increased during exercise in five patients and decreased slightly in one (no 5) After exercise the B P fell to almost the same value as before exercise The plasma volume was reduced in all cases (between 3 and 18 %)

Guanethidine treated patients

As shown in Table III and Fig 3 the effect of short term exercise on B P and plasma volume was much more varied in the guanethidine treated patients than in the above mentioned patient groups During exercise there was a fall in mean B P in four patients (nos 1 3 7 and 8) In no case however was the fall so great that symptoms of cerebral ischemia appeared The B P rose in six cases and in one patient it remained unchanged For the group as a whole there was a small rise in mean B P that was not significant Five to ten minutes after exercise the B P fell in all patients to lower values than before exercise and in seven patients (nos 2 3 6 7 8 9

Table II *Effect of exercise in untreated hypertensive patients*

Pat. no	Sex	Age	Blood pressure (Mean) (mm Hg) Sitting rest	Sitting exercise	Sitting post exercise	Plasma volume (ml) Before exerc	After exerc	Per cent change	Serum sodium (mEq/l) Before exerc	After exerc	Hematocrit (%) Before exerc	After exerc
1	♂	57	200/127 (164)	233/130 (182)	192/128 (163)	4053	3707	(−8.5)	143.0	143.5	40.0	42.0
2	♂	62	172/110 (141)	260/122 (181)	188/108 (148)	2950	2418	(−18.0)	141.5	141.0	47.0	50.5
3	♀	64	197/122 (160)	252/127 (190)	200/130 (165)	2219	2052	(−7.5)	145.0	145.0	45.0	46.0
4	♂	6	152/100 (126)	203/88 (146)	117/88 (98)	3743	3624	(−3.2)	146.0	145.0	46.0	46.0
5	♂	50	175/110 (143)	198/83 (142)	170/107 (139)	2866	2749	(−4.1)	144.0	144.5	44.0	45.0
6	♂	58	207/120 (164)	230/130 (180)	193/130 (162)	3430	3087	(−10.0)	142.0	143.5	40.5	41.0
Mean			180/113 (147)	225/112 (169)	172/113 (143)	3401	3035	(−10.4)	143.6	143.7	43.8	45.1
Change ±S.E.M.			20±3 ($p<0.001$)			164±29 ($p<0.001$)						

Mean B.P. = (systolic + diastolic)/2

and 11) the fall was so great that symptoms of hypotension in the form of dizziness and lethargy developed. Five of these patients (nos 6, 7, 8, 9 and 11) were very close to fainting. Mean plasma volume did not change during exercise, but there was rather pronounced individual variation (Table III, Fig 3). Plasma volume increased 8.4% in patient 1 and mean B.P. fell 11 mm Hg. In patients 2 and 10 the plasma volume decreased 5 and 7%, respectively, while there was a rise in mean

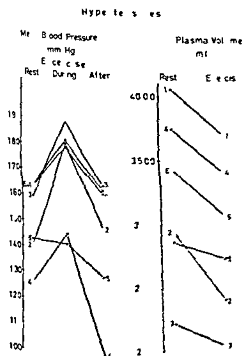


Fig 2. The effect of short-term exercise on mean blood pressure and plasma volume in six patients with untreated hypertension.

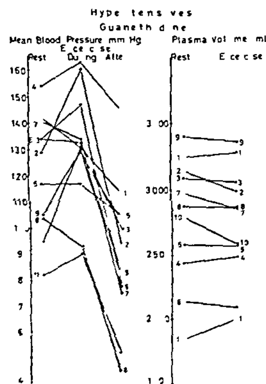


Fig 3. The effect of short-term exercise on mean blood pressure and plasma volume in 11 hypertensive patients treated with guanethidine.

Table III *Effect of exercise in guanethidine treated hypertensive patients*

Pat no	Sex	Age	Blood pressure (Mean)[a] (mm Hg) Sitting rest	Sitting exercise	Sitting post exercise	Symptoms[b] post exercise	Plasma volume (ml) Before exercise	After exercise	Per cent change
1	♀	58	180/103 (142)	168/93 (131)	138/90 (114)	–	1850	2007	(+8 4)
2	♂	55	167/90 (129)	212/108 (160)	110/77 (94)	+	3151	2992	(–5 1)
3	♂	55	158/110 (134)	158/107 (133)	117/83 (100)	+	3092	3064	(–0 9)
3	♀	50	203/105 (154)	213/113 (163)	183/107 (145)	–	2442	2496	(+2 2)
5	♀	34	140/93 (117)	143/90 (117)	130/80 (105)	–	2585	2575	(–0 3)
5	♀	49	160/108 (134)	193/100 (147)	88/67 (78)	+ +	2142	2100	(–2 0)
7	♂	61	175/113 (141)	168/100 (134)	85/65 (75)	+ +	2975	2859	(–3 9)
8	♀	52	122/85 (104)	110/75 (93)	50/40 (45)	+ +	2877	2877	(–0 0)
9	♂	57	123/87 (105)	155/103 (129)	97/68 (83)	+ +	3408	3368	(–1 2)
10	♂	59	110/80 (95)	163/97 (130)	127/80 (104)	–	2793	2595	(–7 0)
11	♂	42	100/63 (82)	110/70 (90)	63/40 (52)	+ +	3257	3289	(+1 0)
Mean			149/94 (127)	163/96 (130)	133/75 (94)		2779	2748	(–0 8)
Change ± S E M				+8±5 ($p>0$ 1)				–31±30 ($p>0$ 1)	

[a] Mean B P = (systolic + diastolic)/2 [b] – = no symptoms + = dizziness and lethargy + + = incipient syncope

B P of 31 and 35 mm Hg In the other patients only small changes in plasma volume (–3 8 to +2 2 %) were seen Even though the greatest changes in plasma volume were accompanied by an opposite change in B P there was no relation ship between the magnitude of the changes in plasma volume compared to the changes in B P In general a decrease in plasma volume was ac companied by a rise in hematocrit and vice versa however it is surprising that the large decrease in plasma volume of 7 % in patient 1 did not result in a rise in hematocrit Serum sodium did not change during exercise

DISCUSSION

The present investigation has shown that there is a decrease in plasma volume after short term exercise in normotensive and untreated hyper tensive patients This finding agrees well with what other investigators have reported Thus Ny lin (14) Ebert et al (5) and Saltin (18) found a decrease in plasma volume between 7 and 20% in normal individuals after short term exercise Kaltricher et al (10) demonstrated a decrease in plasma volume in six normal individuals of 2 to 25 % after ten minutes exercise and a small de crease in patients with compensated heart disease In one hypertensive patient they found a decrease in plasma volume of 8 2 % and concluded that increased intracapillary pressure during exercise produced a leakage of fluid from the vascular space On the other hand Iseri et al (8) postu lated that the decrease in plasma volume results from a transudation of water from the capillaries to the working muscle cells due to the increased osmotic pressure in these cells during exercise They supported this theory with among other things the observation that short term muscular exertion in recumbent as well as sitting patients with compensated heart disease not only resulted in a significant decrease in plasma volume but also an increased plasma osmol and sodium con centration Iseri et al (8) found in addition a decrease in plasma volume of 12 % in resting pa tients made to stand from a recumbent position This decrease in plasma volume was considered to be due to transudation of a plasma ultra filtrate from the vascular space because of an increased orthostatic pressure as no change in plasma osmol and sodium concentration occurred and the decrease in plasma volume was prevented if these patients were made to stand in deep water There was no change in serum sodium concentration during short term exercise in the present investigation and therefore it was not possible to verify Iseri et al s theory that the decrease in plasma volume during exercise is the

odium ч.l)		Hematocrit ( )	
terc.	After exerc	Before exerc	After exerc
47 0	146 0	41 0	40 0
40 0	141 0	46 0	47 0
50 0	146 5	42 0	44 5
34 0	142 0	41 0	40 0
.0 0	14 0	43 0	45 0
0	149 0	39 0	39 0
	141 0	48 5	49 0
	145 0	42 0	4 0
0	144 0	45 0	46 0
19 5	141 0	45 0	45 0
15	144 0	40 0	39 0
39	143 8	43 0	43 9

result of an increased osmotic activity in the working muscle cell It may be presumed however that the decrease in plasma volume demonstrated in this investigation in both normotensive and untreated hypertensive patients is the result of a loss of isotonic fluid due to increased intracapillary pressure in the contracting hyperemic muscles

The mean plasma volume and mean B P remained unchanged in the 11 guanethidine treated patients during exercise The blood pressure even fell in four Five to ten minutes after exercise the B P fell so greatly in seven patients that symptoms of cerebral ischemia developed and five were very close to fainting Similar observations were first recorded by Leisman et al in 1959 (11) and since then by several other investigators (3 4 9 13 19) Dollery et al (3 4) however noted a rise in B P 2 to 3 minutes after exercise to a level higher than that recorded before exercise even though they too found a fall during exercise No such rebound phenomenon was seen in the present investigation the reason most likely being that Dollery et al carried out their studies on recumbent subjects while the present investigations were performed on sitting subjects A fall in B P during exercise in guanethidine treated patients is either due to a fall in cardiac output and/or a decrease in peripheral vascular resistance It is well known that guanethidine given intravenously (1) as well as orally (15) causes a fall in cardiac output in recumbent resting patients Taylor and Donal (19) and Dollery et al (4) however found that guanethidine administered during exercise did not affect the ability of the heart to increase cardiac output even if the B P remained unchanged or even fell pronouncedly That must mean that exercise produces a very marked decrease in peripheral vascular resistance The fall in B P after exercise is apparently the result of a fall in cardiac output toward the resting level while at the same time the peripheral vascular resistance remains strongly reduced It is of course possible that the reason why short term exercise does not cause a reduction in plasma volume in guanethidine treated patients is that the vasodilative action of guanethidine reduces peripheral vascular resistance and thereby decreases intracapillary pressure in contracting muscle

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TREATMENT OF HEART BLOCK FROM A CLINICAL VIEWPOINT

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Abstract In a material of 85 patients with total heart block treated with artificial pacemakers a review of the etiology shows that most of the patients suffered from a diffuse myocardial fibrosis Only in three patients was coronary artery disease present In this material there are also many patients with aortic valvular disease and four with previous toxic diphtheria Most of the patients had atrioventricular block Eight patients had sino-atrial block

Adams Stokes attacks were the predominant indication for cardiac pacing Only a few patients had pacemaker implantation on account of congestive heart failure or vertigo

Various modes of cardiac pacing have been used At the present time intracardial pacemaking is the method of choice and an increasing number of on-demand pacemakers are now applied This is of paramount importance in patients with competition between sinus rhythm and pacemaker rhythm Four such patients were seen in this material Three died suddenly of cardiac standstill in the immediate postoperative period One was successfully defibrillated

The treatment of patients with sino atrial or atrioventricular heart block with cardiac pacemakers has made it possible to sustain life in patients who previously ran a high risk of developing Adams Stokes attacks with cardiac standstill and death This treatment however still offers several problems as to the maintenance of an adequate heart rate The problems are partly technical with respect to type of pacemaker and method of applying the cardiac electrodes There are also some medical problems in these patients as to the etiology of their heart block the problem of interference between pacemaker and sinus rhythm in those patients who eventually return to sinus rhythm and to the mode of adequate control in these patients

MATERIAL

Since 1960 85 patients have been treated with an artificial pacemaker at the University Clinic Rikshospitalet, Oslo The material consists mainly of patients with chronic heart block as up to now very few patients with acute myocardial infarction are admitted to this hospital We also have seen very few patients with acute heart block following cardiac surgery This we feel is due to the technical skill of our cardiac surgeons As will be seen from Table I there is a preponderance of men 69 men and 6 women The majority of the patients are in the age group 60 to 70 years The youngest patient was 15 years and the eldest 86 years old

As to the etiology of the heart block (Table II) the opinion has changed during the latest years Previously it was supposed that most cases of chronic heart block were due to coronary arteriosclerosis with occlusion of coronary arteries producing ischemia of the atrioventricular node Pathological studies by Lenegre (5) and by Lev (6) have however shown that in most cases of chronic heart block there is only minimal or no atherosclerosis of the coronary vessels An unspecific fibrosis is found in the conduction system producing interruption of atrioventricular conduction This viewpoint has been supported by pathological studies carried out in our material Of 17 patients who have died autopsy has been made in ten of them In nine patients diffuse myocardial fibrosis was found in the inter ventricular septum involving the atrioventricular node Only in one patient who died from acute myocardial infarction with atrioventricular block was occlusion of coronary arteries found This patient had an old occlusion of his right coronary artery and a fresh occlusion of the descending branch of the left coronary artery Acute heart block during myocardial infarction is mostly produced by occlusion of the right coronary artery as the atrioventricular nodal artery usually arise from this artery in 85 of the patients (3) Coronary angiography has been carried out in three of our patients All had patent coronary arteries Fig 1 shows a characteristic specimen

Table I *Age and sex*

	Age <20	21–30	31–40	41–50	51–60	61–70	71–80	>80	Total
Men	1	0	3	3	10	26	13	3	69
Women	0	0	1	5	4	9	7		26
	1	0	4	8	14	35	20	3	85

of the myocardium in the region of the atrioventricular node from a case with permanent heart block As we see there is a diffuse unspecific fibrosis of the myocardium

Congenital heart block is not represented in the present material During the same period we have seen seven cases with congenital heart block ranging in age from 1 to 23 years But in none of the cases was there an indication of cardiac pacemaking It is always difficult to tell when one sees a child with complete heart block whether it is congenital or due to a previous myocarditis The patients have no symptoms from their heart block It is discovered accidentally mostly at school health examination The heart rate in congenital heart block is usually higher than in older patients most commonly around 50 There is also no bundle branch block in congenital heart block But as we shall see later nodal origin of the idioventricular beat may also be found in acquired heart block Previously congenital heart block was considered to be combined with other cardiac defects especially ventricular septal defect It is now well known that the systolic murmur along the left sternal border which previously supported a clinical diagnosis of ventricular septal defect is due to the large stroke volume in heart block producing an ejection systolic murmur We have carried out right heart catheterization in two patients who previously were supposed to have ventricular septal defect No septal defect was found at catheterization (9)

Table II *Etiology of heart block*

Myocardial fibrosis	57
Aortic valve disease	13
Rheumatic infection	5
Diphtheria	4
Coronary heart disease	3
Boeck s sarcoid	1
Lipoidosis	1
Surgical heart block	1



Fig 1 Microphotograph of myocardium in the region of the atrioventricular node showing extensive fibrosis with varying size of myocardial fibers and an increased number of lymphocytes H E ×100

For many years especially since the work by Yater and Cornell (10) it has been known that calcification in the aortic valve region and pars membranasea produces permanent heart block As we see a surprisingly high percentage of our patients had aortic valvular disease It may also be mentioned that five of Stokes seven patients had aortic valvular disease (7)

Only one patient had acute myocardial infarction A further two patients had angina pectoris and we feel that in these three patients the heart block was due to coronary atheromatosis

A history of rheumatic infection was found in five patients It is of course difficult to tell whether their heart block is due to rheumatic myocarditis as at the present time there were no rheumatic stigmata

Table III *Cardiac symptoms and indications for cardiac pacin*

	Cardiac symptoms	Indication for cardiac pacing
Adams Stokes attacks	77	77
Congestive heart failure	42	7
Verti o	30	1

A surprisingly large number six patients had a history of diphtheria many years ago In four of the patients there was a history of severe toxic diphtheria and we feel that in these four patients their heart blo k is due to their previous diphtherial myocarditis

We found two rare cases of complete heart block one case of Boeck s sarcoid with supposedly myocardial infiltration by sarcoid tissue and one case of lipoidosis of the type Spielmayer Voot, a pe uliar form of systemic lipoidosis which in this young boy of 13 years also infiltrated his myocardium

Surgical heart block is only represented by one patient in our material The patient had implantation of a mitral prosthesis After the operation there was a total atrioventricular block which made it necessary to apply a cardiac pacemaker The patient died however from myocardial failure

CARDIAC PACING

As we see from Table III the indication for implantation of a cardiac pacemaker has mostly been Adams Stokes attack in 77 patients In seven patients the indication was congestive heart failure due to the slow ventricular rate in patients with a poor myocardium Only in one patient was disabling vertigo the indication for pacemaking Forty two patients had congestive heart failure on admission and 30 complained of dizziness

Table IV *Electrocardiographic findings*

Sino-atrial block	8
Atrioventr cular block	77
Nodal rhythm	30
Bundle branch block	
Right	18
Left	22
Left and right	15
Atrial flutter or fibr llation	7

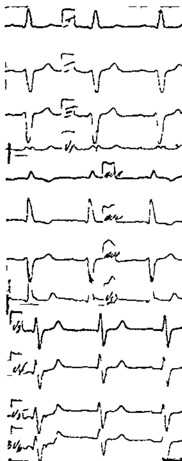


Fig 2 ECG from a patient with total A V block showing left bundle branch block in limb leads and right bundle branch block n precordial leads

Table IV shows that not only atrioventricular block but also sino atrial block may produce Adams Stokes attacks and make it necessary to implant a cardiac pacemaker

In most of the patients there was a bundle branch block either right or left or combined right and left In 30 patients however the idio

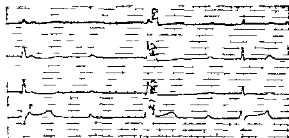


Fig 3 ECG from a patient with total A V block, showing nodal origin of the idioventricular beat

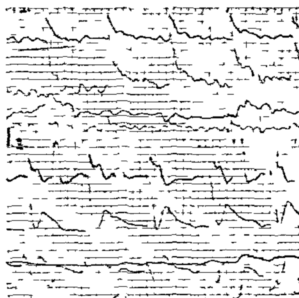


Fig 4 Upper four ECG leads showing ventricular fibrillation in a patient with implanted cardiac pacemaker Lower four leads showing fixed rate cardiac pacing after *DC* defibrillation.

ventricular beat originated in the atrioventricular node with a normal QRS duration (Figs 2 and 3) Seven patients had atrial flutter or fibrillation combined with their total heart block. These patients are of special interest in the discussion about P-synchronous pacemaker as of course that type of pacemaker should not be used in these patients We shall return to that problem later

In a certain number of patients presenting with total heart block and Adams Stokes attack sinus rhythm eventually returns That has been the experience in 12 of our patients The return of sinus rhythm poses the problem of interference between pacemaker rhythm and sinus rhythm with the possibility that the pacemaker impulse may fall in the vulnerable period of the cardiac cycle thus producing ventricular fibrillation and death. Sowton (7) had made a calculation showing that 5% of the stimuli that is approximately 3000 impulses every 24 hours will fall within the vulnerable period Only in a minority of patients will the interference produce ventricular fibrillation. The danger of ventricular fibrillation is highest in the immediate post-operative period Bilitch and associates (1) in 40 patients with pacemaker implantation observed five cases of ventricular fibrillation with death in the immediate post-operative period Sowton (7) similarly observed four deaths among 60 patients treated with an artificial pacemaker all in the immediate post-operative period We have seen one patient with ventricular fibrillation in the post operative period successfully treated with defibrillation (Fig 4) The patient is still alive Three further patients died from cardiac standstill in the post-operative period Although there is no electrocardiographic verification we feel that death was due to ventricular fibrillation in these patients In the follow up there is however no excess mortality in patients with competitive rhythm Of 17 deaths only one patient was known to have competitive rhythm while of the living 69 patients 11 had interference between sinus rhythm and pacemaker rhythm at the latest control Of these 11 patients pacing has been suspended in three

Some patients with P-synchronous pacemakers have developed atrial fibrillation or flutter making it desirable to convert their rhythm to sinus rhythm In three patients DC conversion has been carried out Defibrillation was successful in two of the patients A fourth patient has not been referred for atrial defibrillation

Table V shows the mode of cardiac pacing Twenty nine intracardiac pacemakers have been applied 20 fixed rate epicardial pacemakers 33 P synchronous epicardial pacemakers and three on-demand pacemakers

There have been technical problems with all types of cardiac pacemakers which I shall not deal with in detail I may say merely that from a medical viewpoint it seems most desirable to use intracardiac pacemakers as thoracotomy prolongs the hospitalization period and in some patients produces pleural exudate which has to be treated In one patient with an intrathoracic P synchronous pacemaker we have observed a prolonged post pericardiotomy syndrome necessitating steroid treatment up to now for eight months

The life of the batteries has varied greatly in this material On an average the life period of

Table V *Mode of cardiac pacing*

Intracardial	29
Epicardial	20
P synchronous	33
On-demand	3

Table VI *Cause of death*

Congestive heart failure	5
Sudden death	7
Cerebro-vascular disease	2
Carcinomatosis	1
Myocardial infarction	2
	17

the batteries was 16 months in two patients up to 30 months and in one patient 39 months

Repositioning of the intracardiac electrode or repair of broken leads has been carried out altogether 30 times in this material

As mentioned 17 of our patients have died Table VI shows the cause of death in these patients Five patients have died of congestive heart failure which developed in spite of adequate cardiac pacing Seven patients died suddenly Three of these patients died in their home the first patient one month after change of his pacemaker battery There was no competitive rhythm The second patient was known to have a heart rate of 120 shortly before death She may have represented the runaway phenomenon of the battery The third patient had pacemaker failure with a heart rate of 40 The fourth fifth and sixth patients died in the immediate post operative period of cardiac standstill There was known to be interference between pacemaker and sinus rhythm but there was no electrocardiogram from the period of cardiac standstill The seventh patient died on the operating table of cardiac standstill during the introduction of the endocardial electrode

Two patients died of myocardial infarction two of cerebrovascular disease and one of metastatic carcinomatosis

Fig 5 shows the survival period in our material As we see it compares favorably with earlier results as presented by Sowton (8) There is a

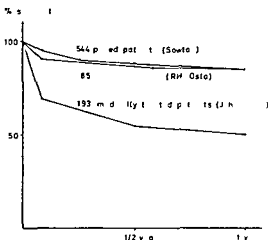


Fig 5 Survival curve for the first year in the present material compared to Johansson s medically treated material and Sowton s material collected from the literature

distinct difference between the survival period in patients with heart block treated with cardiac pacemakers and the natural history of heart block as demonstrated by Johansson (4) with only 50 survival after one year It should however be noted that Johansson s material includes a large number of patients with heart block during myocardial infarction

Should all patients with complete heart block be referred for implantation of cardiac pacemakers? Or is it possible on medical grounds to select patients who may be treated with drugs? To illustrate this problem we have reviewed the history of 20 patients who during this same period have been studied in the Medical Department for treatment of their heart block and who were not referred for cardiac pacing The age distribution of this material shows a range from 15 to 73 years with a mean age of 54 5 years against 63 3 years in the pacemaker material It is remarkable that the largest number of these patients were women 14 against six men a reversal of the

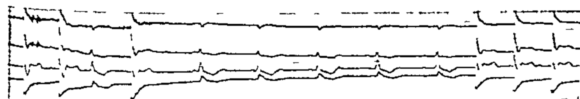


Fig 6 Cardiac standstill produced in a patient with on demand pacemaker by moving an electric razor across his chest

sex ratio in the pacemaker material All of them had total heart block two sino-atrial block and 18 atrioventricular block Only half of the patients had experienced Adams Stokes attacks The duration of the disease varied greatly One patient was known to have had heart block for 28 years Only one of these patients has died This was a woman of 50 years old who died suddenly one year after admission to the department The remaining patients are alive and some of them are leading an active life Two patients are now known to be in sinus rhythm

Although the mortality is small in this selected medically treated material we feel that the indications for cardiac pacemaking should be widened When a patient with total heart block presents with a history of Adams Stokes attack congestive heart failure or dizziness he should be referred for pacemaker implantation

As to the mode of cardiac pacing we feel that intracardiac pacemaker should be the treatment of choice and we also feel that on-demand pacemakers should be used more frequently With on-demand pacemaker one avoids the problem of interference between pacemaker rhythm and sinus rhythm in those patients who later return to sinus rhythm We are aware of the present technical problems with on-demand pacemakers One such problem is shown in the next figure (Fig 6 (2)) which shows that the patients electric shaver produces cardiac standstill Other kinds of electric noise like automobile engines are also known to interfere with the function of on-demand pacemakers but we hope that these technical problems ill be solved in the near future

How often should patients with cardiac pacemakers be controlled? We instruct the patients to seek medical advice immediately if they note disturbances of rhythm and especially if they experience tachycardia The patients are advised to have an electrocardiogram taken every third month. As our patients are referred from the whole country most of them visit their regional medical department Only a few of the patients are seen at the Rikshospital As mentioned our follow up study h is shown that pacemaker failure was probably the cause of death in two patients

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EFFECTS OF GLUCOSE INFUSIONS ON ADIPOSE TISSUE LIPOGENESIS IN MAN

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Abstract Incorporation of label from glucose 1 C with different concentrations of non labelled glucose and aceta U-C with or without added non labelled glucose into carbon dioxide fatty acids and glyceride glycerol was measured in human subcutaneous adipose tissue taken from patients with clinically "well-controlled" juvenile diabetes mellitus or from controls The effects of infusion of glucose in the control and glucose plus insulin in the diabetic patients on the day preceding adipose tissue biopsy and of the addition of insulin or citrate in vitro were investigated In normals glucose infusions before biopsy caused an increase in in vitro response to insulin of carbon dioxide formation from labelled glucose and an increased incorporation of label from acetate (with 10 mM glucose) into carbon dioxide and fatty acid In diabetic patients such an increase of label in carbon dioxide and fatty acids was found with labelled glucose (1 mM) and the resulting values were as high as those of the normals who had not received glucose before sampling, but they did not reach the values found in normals who had been given glucose A similar remaining deficiency after glucose infusion was found in the diabetic tissues in incorporations of labelled acetate (with 10 mM glucose) the values being as high as those of normals without preceding glucose infusions but lower than those of glucose treated normals The variability of insulin response of human adipose tissue in different metabolic situations may indicate that adipose tissue glucose uptake contributes to determining glucose tolerance in man Furthermore adipose tissue in man may be an important site for glucose lipogenesis during abundance of glucose while in other metabolic situations the liver may well be of relatively greater importance for lipogenesis

Lipogenesis in experimental diabetes is severely depressed as shown originally by Stetten and Boxer (16) This abnormality has later received much attention in the experimental animal In the human with poorly controlled diabetes mellitus Awai and co-workers have demonstrated a decreased fatty acid synthesis in blood (1) and blood platelets (2)

In adipose tissue a number of studies of fatty acid synthesis have been performed in experimental diabetes (17) Goldrick and Hirsch (9) recently demonstrated a decrease of lipogenesis from glucose and acetate during fasting in human adipose tissue Also in the human with diabetes mellitus glucose and acetate lipogenesis in adipose tissue is severely depressed (3 13) Such a depressed lipogenesis seems to be at hand also in diabetes mellitus which is well controlled from the clinical point of view (3) A similar phenomenon has previously been observed with acetate incorporation into plasma lipids in clinically well controlled diabetes mellitus (12) Patients with such a degree of control of their diabetes mellitus are however by no means metabolically normal exhibiting raised concentrations of blood glucose and plasma free fatty acids The lipogenesis abnormality then could be a sign of a remaining insulin deficiency

In order to investigate whether a remaining insulin deficiency was indeed the cause of the lipogenesis abnormality and thus could be normalized patients with diabetes mellitus were given glucose and extra insulin infused intravenously preceding investigation of adipose tissue lipogenesis

METHODS

Eleven patients with diabetes mellitus of the juvenile type were investigated during hospitalization for different routine investigations concerning diabetes complications. None was under poor control all had blood glucose values on the day preceding adipose tissue biopsy or preceding glucose plus insulin infusion of less than 250 mg per 100 ml blood, and free fatty acids of less than 1 40 microequivalents per liter plasma None had

ketonuria Seven of these patients were investigated with out glucose infusion They did not receive insulin on the day of biopsy Four diabetic patients were investigated after the intravenous infusion of 300 g of glucose in a 15 solution containing 74 units of insulin (Standard Insulin Vitrum Stockholm, Sweden) given during the day before biopsy On that day they also received their usual insulin dose In these patients blood glucose was followed at 4 hour intervals before biopsy and found constantly to be below 248 mg per 100 ml in two of the patients, and below 150 in two One of the latter patients required extra carbohydrate perorally during the night before biopsy because of hypoglycemia.

Thirty three patients operated upon for different abdominal diseases mainly gall bladder disease and peptic ulcers served as controls Twenty of these were investigated without preceding glucose infusion Thirteen patients received 300–400 g of glucose in a 15 solution but no insulin on the day before operation None of these patients had any disease of known importance for the study

None of the patients diabetics or controls were infected or had lost weight in the period immediately before biopsy None was more than 20 above ideal weight (15) Therefore it was not considered necessary in the present work to express results on a basis of adipose tissue cell number (cf 3 13) All infusions were finished five or more hours before sampling.

Subcutaneous adipose tissue biopsies were taken in the morning after overnight fast In the diabetic patients this was done under local anesthesia (1 Lidocain without epinephrine Astra Sodertalje Sweden) injected subcutaneously in a rhomboid figure and under the abdominal muscular fascia From the controls subcutaneous or omental adipose tissue was taken at the beginning of operation Anesthesia followed routine procedures viz basal anesthesia with morphine or its analogues before operation, and during operation a combination of Evipan (Sodium hexobarbital Bayer Leverkusen West Germany) nitrous oxide oxygen and succinylcholine Lipogenesis was found to be unaffected by such anesthesia when the comparison was with tissues from patients operated on for inguinal hernia under local anesthesia The tissue was kept at room temperature in Krebs-Ringer bicarbonate buffer with 4 bovine serum albumin (Fraction V Armour Eastbourne England) pH 7 4 and immediately brought to the laboratory and processed

With the aim of minimal handling, pieces of 100–700 mg weight were taken from the center of the biopsy and approximately 0 5 g of these were incubated in each flask after weighing on a torsion balance Incubation during 2 hours was performed in 3 ml Krebs Ringer bicarbonate albumin solution in 50 ml cylindrical siliconized tubes Gas phase was 95 O_2 – 5 CO_2. Incubation flasks were placed in a 37 C waterbath and shaken at 100 cycles per minute

In the experiments in which glucose concentrations were varied the incubation conditions were the following Two flasks, each containing 1 25 5 0 20 0 and 80 0 m*M* final glucose concentration, and two flasks with 20 0 m*M* without tissues as control were incubated A similar series was also incubated with 1000 μU/ml of insulin (Crystalline bovine plus pig insulin Nordisk Insulin Gentofte Denmark) All contained approximately 750 000 cpm of glucose 1 C (CFA 204 The Radiochemical Centre Amersham, England)

In the experiments in which different groups of patients were compared three series of flasks were incubated The first series contained glucose 1 C and unlabelled glucose to a final concentration of 1 0 m*M* Two flasks were incubated without further addition, two with 1000 μU/ml of insulin and two without tissues as control

A second series contained approximately 500 000 cpm of acetate U C (CFA 229 The Radiochemical Centre) and unlabelled sodium acetate to a final concentration of 10 m*M* This series also contained unlabelled glucose to a final concentration of 10 m*M* Flasks with no further addition with insulin and without tissues were incubated as in the first series

A third series was identical to the second except that no glucose was present

In the diabetic patients not treated with glucose insulin infusions series two and three were not performed

In the experiments with glucose acetate glucose or acetate incubations citrate (10 m*M*) was added to two flasks in all patients except the diabetics who did not receive extra glucose plus insulin

After incubation for two hours 0 3 ml of 1 *N* sulfuric acid was injected into the incubation medium and carbon dioxide was collected during 4 hours in 0 3 ml Hyamin 10 X (Packard La Grange Illinois USA) injected into glass beakers suspended in steel wires Lipids were extracted in 15 ml chloroform methanol 2 1 (v/v) according to Folch et al (7) An aliquot of the final chloroform phase was evaporated saponified at 60 C for one hour in an excess of potassium hydroxide acidified and fatty acids were extracted in heptane Carbon dioxide total lipids and fatty acids were then counted in 10 ml of 0 4 of 2,5-diphenyloxazole (Packard) and 0 01 of *p* bis 2 (5 phenyloxazolyl) benzene (Packard) in toluene in a Packard Tri-carb liquid scintillation counter (Packard) Glyceride glycerol label was obtained as the difference between label in total lipids and in fatty acids Incorporation of radioactivity into the measured products was calculated as glucose or acetate from the counts obtained and the original specific activity of the incubation medium

Significance of differences was tested by Student s *t* test

RESULTS

The results of incorporations of radioactivity from glucose 1 ^{14}C at different glucose concentrations are set out in Fig 1 Without insulin addition most label was found in glyceride glycerol slightly less in carbon dioxide while label in fatty acids amounted to between 7 and 17 % of total Carbon dioxide labelling seemed to vary with medium glucose concentration more than did glyceride glycerol and particularly more than fatty acids

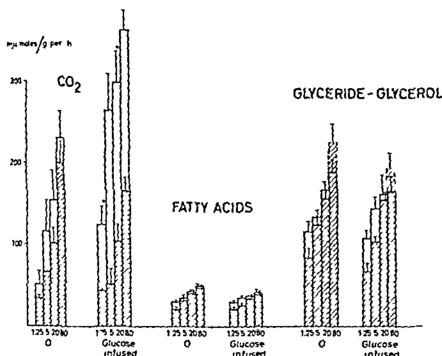


Fig 1 Incorporation of label from glucose 1 ¹⁴C at different glucose concentrations (m*M*) into carbon dioxide fatty acids and glyceride glycerol in controls who had not received glucose (=0) ($n=8$) and controls who had received glucose ($n=7$) *Striped columns* basal values *Open columns* values after addition in vitro of 1000 μU ml of insulin * = $p<0.05$ * = $p<0.01$ * = $p<0.001$ Comparisons between basal and insulin results Values are means ±S E M

labelling Insulin addition in vitro caused a significant increase in incorporation of label into carbon dioxide at 1 25 and 5 mmolar glucose concentration and into fatty acids and glyceride glycerol at 1 25 mmolar glucose concentration After infusion of glucose insulin responses were more pronounced particularly incorporation of label into carbon dioxide

Fig 2 gives the results of labelled carbon dioxide formation in different groups of patients With glucose 1 ¹⁴C the controls responded to insulin in vitro apparently more after infusion of glucose like the patients in Fig 1 After glucose infusion the incorporation of label into carbon dioxide in the diabetic tissues was not lower than controls but did not respond to insulin This incorporation was however lower than after glucose infusion to controls ($p<0.05$) Omental tissues showed a high incorporation with apparently no insulin response

With labelled acetate in the presence of glucose tissues from controls who had not received glucose infusions showed somewhat lower values than the other tissues ($p<0.01$ comparison with glucose infused controls without insulin addition) Tissues from controls and diabetics after glucose infusion showed no differences Here as well as with no glucose present omental tissues showed high incorporations

Fig 3 gives the results of incorporation into fatty acids With labelled glucose the controls were not much different with or without glucose infusion Insulin response was present Diabetic tissues showed very low values without insulin response but after infusion of glucose plus extra insulin they rose to the values of the controls and insulin stimulated synthesis Omental tissues showed the highest incorporations

With labelled acetate in the presence of glucose the controls responded to insulin in vitro After glucose infusion the incorporation into fatty acids rose markedly ($p<0.001$) and still responded to insulin Diabetic patients receiving glucose plus extra insulin were not different from controls who were not given glucose but were lower than glucose treated controls ($p<0.01$) Insulin re

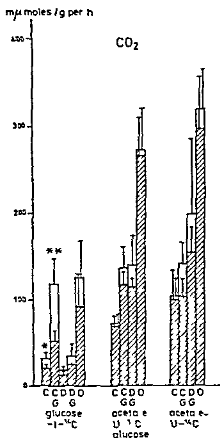


Fig 2 Incorporation of label from glucose 1 ^{14}C (1 m*M*) acetate U ^{14}C (10 m*M*) plus glucose (10 m*M*) and acetate U ^{14}C (10 m*M*) into carbon dioxide in subcutaneous adipose tissues from controls (= C) ($n = 6$) glucose treated controls (= CG) ($n = 6$) diabetic patients (= D) ($n = 7$) and diabetic patients receiving extra glucose + insulin (= DG) ($n = 4$) and in omental adipose tissues from controls (= 0) ($n = 6$) Other symbols as in Fig. 1

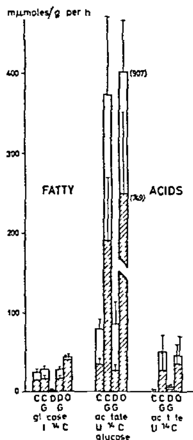


Fig 3 Incorporation of label from different precursors into fatty acids in the same tissues as in Fig 2 Symbols as in Fig 2

sponse was present Omental tissue showed very high incorporations but apparently no insulin response Without glucose some synthesis from acetate occurred in glucose infused controls and omental tissues and very little in the tissues from diabetics who had received glucose plus extra insulin.

Glyceride glycerol labelling (Fig 4) was similar in all subcutaneous tissues whether diabetic or not or whether obtained from patients who were glucose treated or not. Insulin response was present except in the diabetic patients who had received glucose plus insulin Also here omental tissues showed high incorporations

Addition of citrate to the incubation flasks did not show any effect.

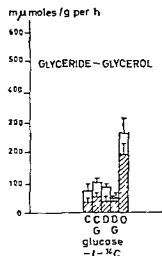


Fig 4 Incorporation of label from glucose 1-^{14}C into glyceride glycerol in the same tissues as in Fig 2 Symbols as in Fig. 2

Since omental tissues showed by far the largest interindividual variations (cf SEM of Figs 2 3 and 4) the differences between basal and insulin stimulated metabolism were analysed pairwise A relatively small stimulatory effect of insulin could then be demonstrated for glucose incorporations in carbon dioxide ($p < 0.05$) fatty acids ($p < 0.05$) glyceride glycerol ($p < 0.01$) and acetate incorporation in fatty acids ($p < 0.05$)

DISCUSSION

Cahill et al (5) pointed out that the incorporation of glucose label into glyceride glycerol in relation to the sum of all incorporations is maintained relatively intact in rat adipose tissue when glucose uptake is decreased suggesting that this pathway assumes first priority In Table I the relative part of glyceride glycerol labelling of the sum of label in carbon dioxide fatty acids and glyceride glycerol has been calculated Glycogen is not included in the sum since it was not measured but this probably causes only a minor error assuming that glycogen labelling is as small in human tissues as in the rat epididymal fat pad (cf 14) The values of Table I demonstrate that in human adipose tissue the relative part of incorporation of label from glucose 1 ^{14}C into glyceride glycerol is usually higher than in rat adipose tissue It varies in the manner that a lower relative incorporation occurs in conditions of a relative abundance of glucose and a high relative incorporation for a relative lack of glucose as in the diabetic tissues and tissues from fasting controls with low in vitro glucose concentration These results indicate that as in rat adipose tissue (5) glyceride glycerol formation assumes first priority also in human adipose tissue

The low glucose concentration employed (1 0 m*M*) in the comparative studies between different groups of patients was chosen because of the previous observation that lipogenesis is stimulated by insulin in vitro at this low concentration (4) With this low glucose concentration considerable dilution of glucose from the medium with intracellular glucose or glucose related metabolic pools seems probable however particularly after preceding glucose infusions Dilution to an unknown extent must also have occurred in the experiments with labelled acetate This makes interpretations of the data difficult from the quantitative point of view

Table I *Incorporation of label from glucose 1 ^{14}C into glyceride glycerol as percentage of total recovered label in measured metabolites in human subcutaneous or omental fat in different conditions*

Comparison with the rat epididymal fat pad data taken from literature (5 14)

Glucose concentration (m*M*)	Insulin (μU/ml)	Glyceride glycerol ^{14}C ( )
Human		
Normals glucose infused		
1 25	1000	41
5	1000	32
20	1000	3[illegible]
80	1000	28
1 25	—	51
5	—	54
20	—	53
80	—	48
Normals fasting		
1 25	1000	59
5	1000	46
20	1000	46
80	1000	39
1 25	—	74
5	—	56
20	—	52
80	—	48
Diabetes glucose infused		
1 0	—	57
1 0	1000	39
Diabetes		
1 0	—	75
1 0	1000	72
Omental tissues		
1 0	—	62
Normals fasting		
1 0	1000	60
Rat		
Fed ad libitum[b]		
1 25	100 000	8
5	100 000	7
0	100 000	5
80	100 000	3
1 25	—	31
5	—	18
20	—	19
80	—	11
Fasting 24 h		
5	—	58
Fasting 7[illegible] h		
5	—	64

Per cent of label in glyceride glycerol fatty acids and carbon dioxide
[b] Calculated from Jeanrenaud and Renold (14)
Calculated from Cahill et al (5)

The severest abnormality in the metabolism of

adipose tissue from juvenile diabetes patients noted previously viz. decrease in fatty acid labelling from glucose 1 ^{14}C (3) was thus possible to correct by measures aimed at increasing adipose tissue glucose uptake in vivo before biopsy viz glucose insulin infusion This was also the case with incorporation in carbon dioxide Furthermore in this situation acetate incorporation into fatty acids in the diabetic tissues was not lower than that in control tissues When comparisons are made with the controls who had received glucose before sampling however a remaining deficiency was found in the diabetic tissues in glucose label incorporation into carbon dioxide and acetate label incorporation into fatty acids These remaining abnormalities might represent differences between controls and diabetic patients requiring a longer time for repair with the aid of increased glucose uptake in vivo in adipose tissue of diabetic patients To what extent the observed differences are caused by varying dilution of radioactive precursors or by a deficient response to insulin of the lipogenesis mechanism is the subject of current studies

Thus even if dilution phenomena always make quantitative interpretations difficult in the present type of studies the insulin response in vitro does not seem to be subject to these errors The insulin response of human adipose tissue in vitro has been much discussed during recent years as recently reviewed by Gries and Steinke (10) We have previously demonstrated that physiological ounts of insulin stimulate human subcutaneous e tissue in vitro and that there is a difference in the magnitude of the response of different metabolic pathways (3) Furthermore certain important determining factors for insulin response in vitro have been pointed out (3 4) The present work shows in addition that the glucose uptake in adipose tissue before the sampling for in vitro studies is important for insulin stimulation in vitro of the incorporation of label from glucose 1 ^{14}C into carbon dioxide and acetate U ^{14}C into fatty acids

The present results show that in man the insulin response of adipose tissue varies with varying abundance of glucose during the time preceding sampling These findings seem to have several potentially interesting consequences The well known effect of diet on glucose tolerance in man (see (8) for review) might be partially explained by this varying capacity of adipose tissue to take up glucose Furthermore due to findings of a small insulin response of human adipose tissue in vitro it has been discussed whether adipose tissue or the liver is the main site for lipogenesis in man (6) The present findings might mean that the dominating part of glucose lipogenesis might occur in adipose tissue or in the liver in different metabolic situations

Human omental adipose tissue has earlier been shown to possess a livelier metabolism than subcutaneous adipose tissue with a considerable ability to synthesize fatty acids particularly from acetate (11) This was confirmed in the present work in which its measured metabolic pathways were even more active than those of subcutaneous adipose tissue from glucose loaded patients

ACKNOWLEDGEMENT

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STOMACH ULCER AND DUODENAL ULCER RUNNING A FATAL COURSE DURING STEROID TREATMENT

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Abst act According to the certificates of death filed with the Dan sh National Health Service 51 gastroduodenal ulcers with a fatal course occurred during steroid treatment of hospitalized patients in the five year period from 1960 to 1964 which is 3 5 of all lethal ulcer cases in the five year period The records pertaining to the patients in question have been reviewed There were 18 males and 33 females The average age was about 65 years More than 50% of the patients suffered from rheumatoid arthritis Steroids were given most often as prednisone over several years the average dose being 15 to 0 m daily The ulcers were often large and most frequently located in the stomach Almost half the pa tents had no dyspeptic symptoms prior to the complica t on of ulcer Histologically 13 out of 30 ul ers were without f brosis No def nite correlation was found be tween, on the one hand the characteristics of the ulcers and on the other the dose of steroids and the duration of treatment

It is generally held that administration of corti costeroids involves the risk of development of re activation of gastroduodenal ulcers Attempts have been made to explain the potential ulcerogenic properties of the steroids partly by an increased secretion of acid and pepsin partly by a weaken ing of the mucosal barrier" Finally the anti inflammatory effect of the steroids which inter feres with the normal structure of tissue has been mentioned (3 8 10)

This problem is however under discussion and in spite of considerable clinical and experimental work no clearcut answer yet exists

MATERIAL

The purpose of the present study was to analyse the course in fatal gastroduodenal ul ers in pat ents, who ha e also been treated with stero ds

By a re iew of the total number of death certif cates filed w th the Danish Nat onal Health Service overing the years 1960 to 1964 for the entire country 5 death certificates were found for patients in whom the cause of death was gastroduodenal ulcers and which al o stat d that the patient was under steroid treatment This must be taken as a minimum figure

The total number of fatal gastroduodenal ulcers in the same period was 1505 and consequently the fatal ul ers in which steroids were involved amounted to 3 5 of the entire group

Fifty-one of the 5_ patients were in ho pital when death occurred One patient who d ed in his home is not included in the material

The hospital records of the patients have been reviewed and on the basis of the collected data an attempt was made to evaluate the importance of the steroid treatment for the appearance of ulcers and for the fatal course

RESULTS

In Table I the age and sex distribution of the material is shown The average age was rather high about 65 years and 37 patients (73 %) were over 60 years of age

Table II shows the disease for which the pa tients received steroids Rheumatoid arthritis is by far the most frequent and comprises more than 50% of the patients As was the case with the remaining diseases it has often been severe and has lasted for many years

Prednisone was the preparation most frequently used and at the time of occurrence of the com plication almost all the patients received this preparation

Seventeen patients had been treated previously with other steroids two with cortisone and the remaining 15 with preparations such as predni solone methylprednisolone fluoxyprednisolone etc

The steroid treatment had often been given over long periods 34 patients (67) were treated for more than one year and of these 24 (47) had been treated for more than three years

Table III shows the size of the steroid dose Steroids other than prednisone have been converted into units of weight equivalent to prednisone Only few patients were given long term doses of 20 mg or more daily the majority received from 10 to 15 mg daily During the crucial period around the time of the complication the dosage was somewhat higher although 24 patients (47%) had still not received more than 15 mg of prednisone daily

As regards 16 patients it was recorded that they presented a cushingoid appearance In four of these osteoporosis had been demonstrated by radiography and in nine cases adrenal atrophy was found at autopsy Another three patients had osteoporosis and eight patients had adrenal atrophy verified by autopsy Hence a total of 27 patients or more than 50% of the material had severe side effects from the steroid therapy which in almost all cases had lasted for more than 3 years

Table I *Age and sex distribution of the patients*

Age (y)	♂	♀
20–29	1	—
30–39	1	–
40–49	—	1
50–59	4	5
60–69	6	14
70–79	6	7
80–89	—	4
Total	18	33

Table II *Survey of underlying diseases*

Diagnosis	♂	♀	Total
Rheumatoid arthritis	9	21	30
Osteoarthritis of various localization	1	2	3
Cirrhosis of the liver or hepatitis	1	4	5
Bronchial asthma	3	—	3
Pemphigus	—	2	2
Lymphatic and myelogenous leucaemia	2	—	2
Haemolytic anaemia	1	—	1
Chronic pyelonephritis	—	1	1
Malignant lymphogranulomatosis	—	1	1
Pancreatic insuloma	1	—	1
Peritoneal carcinoidosis	—	1	1
Mammary cancer with metastases	—	1	1
Total	18	33	51

Table III *Average daily dose of steroids (converted into mg of prednisone)*

mg	Long term dose	Dose at occurrence of complication
0–9	7	4
10–15	23	20
16–45	13	16
≥50	2	6
Not stated	6	5
Total	51	51

Table IV *Location of ulcers*

Duodenum	11
Antrum	15
Lesser curvature	9
Greater curvature	5
Remaining part of the body	2
Cardia	4
Not stated	5
Total	51

Autopsy was performed in 43 of the 51 patients included in the material

Information regarding other ulcerogenic drugs has also been sought but only in 23 case records is it stated expressly that the patient had been treated with salicylates However it must be assumed that this figure is too low since supposedly all patients with rheumatoid arthritis use salicylates In nine patients information was available as to treatment with phenylbutazone preparations or their derivatives and in five of these cases the patient received Butazolidin® at the time of complication

Only eight of the 51 patients had stated that they had had dyspeptic symptoms prior to the institution of the corticosteroid treatment and in only three patients had gastroduodenal ulcers been diagnosed previously Two of these three patients had more than one ulcer at autopsy

A total of 22 patients (43%) had had no dyspeptic symptoms till at the earliest 14 days before the occurrence of the fatal complication of ulcer

In 24 patients (47%) the complication became manifest in the form of perforation in some cases accompanied by haematemesis or melaena and 14 of these perforations were obscured In the remaining 27 patients there was haematemesis

Table V *Size of ulcers*

<1 cm	3
1–<2 cm	7
2–<3.5 cm	12
3.5–<5 cm	10
≥5 cm	6
Not stated	13
Total	51

and in some cases melaena in four patients only a violent melaena appeared

Table IV shows the site of the ulcers and in Table V the size is shown There were three times as many stomach ulcers as duodenal ulcers and on the whole they were rather large 28 (55%) being more than 2 cm in diameter Eleven patients (22) had more than one ulcer and another five patients had one or more superficial erosions in addition to the ulcer

Macroscopical examination of the ulcers showed that fibrosis was not present in 13 cases 17 were fibrous the appearance of the remaining 21 ulcers has not been described Microscopical examination was made infrequently

The stomach ulcers were larger than the duodenal ulcers More often than not they were with out grossly visible fibrosis multiple or associated with erosions The presence of dyspepsia and the tendency to perforation was not determined by the locality Dyspepsia was present with almost equal frequency in small and large ulcers Small ulcers perforated more frequently and the perforations in the small ulcers were obscured more often than in large ulcers

On the whole the non fibrous ulcers were larger than the remaining ulcers and they perforated frequently (10 out of 13) but the perforations were not obscured more often than those occurring in the fibrous ulcers

In the 30 patients with rheumatoid arthritis the ulcer was frequently located in the stomach (duodenum/stomach ratio (d/s) = about 1/4) The five patients with cirrhosis presented duodenal and stomach ulcers with almost equal frequency (d/s ratio = 2/3) and the d/s ratio for the remaining patients suffering from various disorders was about 1/3 In patients with rheumatoid arthritis perforations were obscured less frequently than in the remaining patients which is in accordance with the fact that in this material perforations in the stomach are obscured less frequently than the duodenal perforations

In the present study no correlation was demonstrated between on the one hand the size and duration of the steroid dose and on the other the locality symptomatology number size and appearance of the ulcers The various steroid preparations do not seem to present any differences in respect to the character of the ulcers

Seventeen patients were operated on because of the ulcer and five of these died immediately after the operation (within the first 24 hours) All these 17 patients had received steroid cover in connexion with the operation

In only 17 of the 24 patients with perforated ulcers was steroid cover instituted at the time of occurrence of the complication but six of the seven perforations in connexion with which cover was not established were obscured

Out of the 27 patients with haematemesis and melaena ten were given steroid cover Only one of the patients not treated with steroid cover developed symptoms of adrenal insufficiency

Attempts have been made to estimate the importance of the complication of ulcer in resulting death and it was found that in 44 patients there were no directly competitive causes of death However many of the 44 patients suffered from such severe disorders that the prognosis irrespective of the complicating ulcer was extremely grave As regards the remaining seven patients the relationship between the occurrence of death and the complicating ulcer is not indisputable since concurrently with the complicating ulcer or post operatively conditions developed which might also directly have caused death two patients were in hepatic coma one had pulmonary arterial embolus one cardiac rupture one severe purulent tracheobronchitis and one miliary tuberculosis

DISCUSSION

In the literature it is emphasized that the typical steroid ulcer" is characterized by being very large and deep It is soft almost rubber like with moderate induration of the surroundings and on microscopical examination only slight inflammation However several authors (1 5) have not

been able to demonstrate differences between steroid ulcers and other ulcers in respect of histology locality or dimension

A characteristic feature in the present material is the finding of large stomach ulcers Ulcers without fibrosis were on an average slightly larger than the remaining ulcers Steroid ulcers are stated to be very large but possibly this applies also to other ulcers with a fatal course Exact average sizes are not known and consequently a comparison cannot be made

Previous studies of steroid ulcers have revealed a more frequent localization of the ulcer in the stomach than in the duodenum most often a d/s ratio = 1/2 or 1/3 as against the normal ratio of 5/1 In the present investigation the d/s ratio was found to be 1/3 However frequent localization in the stomach is not a special finding in patients under steroid treatment but applies to all gastroduodenal ulcers with a fatal course according to Causes of Death in Denmark (4) the d/s ratio for ulcers is about 1/3

Often steroid ulcers develop without preceding dyspepsia and perforation is frequently stated to be obscured In patients with ulcers without fibrosis dyspepsia was not present less frequently than in other patients and the perforation was not obscured more frequently To this should be added that development of ulcers without dyspeptic symptoms and obscured perforation of the ulcer gives no lead as to the influence of the steroids on the genesis of the ulcer but only that the anti inflammatory effect of the steroids reduces the pains caused by the ulcer

A tendency to development of complications in particular in the form of perforation is a characteristic feature of steroid ulcers and in the present material 24 patients (47°) with perforation of ulcers were found The non fibrous ulcers perforated particularly often (10 out of 13)

In a previous survey of possible steroid induced deaths in Denmark over the period 1955 to 1959 16 out of 38 cases were caused by ulcers 10 by bleeding and 6 by perforation (6)

During a corresponding 5 year period the perforations according to Causes of Death in Denmark (4) accounted for between 25 and 30 o of all deaths from gastroduodenal ulcers (7) Smith et al (9) have demonstrated that complications may occur unexpectedly in up to 39 of non

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MYOCARDIAL INFARCTION AND WHOLE BLOOD VISCOSITY

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Abstract Based on the observation of increased hematocrit levels in patients suffering from acute myocardial infarction, it has been suggested that rheological factors, especially an increased whole blood viscosity have a *primary* importance for the development of myocardial infarction. For this reason whole-blood viscosity and related components were measured in 25 patients with acute myocardial infarction within the first 24 hours and on the twenty-first day after the onset of chest pain On the first day the hematocrit did not differ significantly from those of a control group of healthy subjects of similar age and of the same sex The average whole blood viscosities were significantly higher in patients with myocardial infarction on the first day Simultaneously the concentration of plasma fibrinogen and alpha₂-globulin was significantly increased On the twenty first day of the infarction the average hematocrit values and whole-blood viscosities were significantly decreased The changes in the rheological properties of blood suggest that an acute injury response is operative in acute myocardial infarction

The etiology of myocardial infarction is complex and undoubtedly involves multiple factors In a series of papers Burch and De Pasquale (3–6) have demonstrated that both males and females suffering from acute myocardial infarction have hematocrit levels which are significantly higher than those of control subjects without ischemic heart disease Based on these findings Burch and De Pasquale suggested that rheological factors especially an increased whole blood viscosity have a *primary* importance for the development of myocardial infarction

Recently it has been demonstrated that any larger tissue injury may be followed by rapid changes in the flow properties of blood Bergentz and co-workers (2) and Gelin (8) have both clinically and experimentally demonstrated that a tissue injury within a few hours is followed by an increase in hematocrit and in the viscosity of whole blood Subsequently (within the first 24 hours) an increase in the concentration of alpha globulin and fibrinogen takes place

It is possible therefore that the changes which Burch and De Pasquale registrated could be considered coincidental with the myocardial infarction rather than a causative factor thereof

In order to elucidate this problem we have examined the hematocrit whole blood viscosity and other parameters related to rheology in patients suffering from acute myocardial infarction during the first 24 hours of the acute onset.

OUR INVESTIGATIONS

The material consists of 25 patients with acute myocardial infarction, 20 males and five females The diagnosis of myocardial infarction was established by classic electrocardiographic changes and increased levels of serum glutamic acid-oxaloacetic acid transaminase Special attention was paid to the exclusion of patients who in addition to myocardial infarction suffered from other diseases which in themselves could give rise to changes in whole blood viscosity The control material consists of 30 healthy subjects 15 males and 15 females in the same age range (Table I) all without signs of cardiovascular disease

Blood samples were obtained in the morning from healthy control subjects and from hospitalized patients with acute myocardial infarction within the first 24 hours and on the twenty first day after the onset of chest pain All samples were analyzed for hematocrit total serum protein (Goldberg Refractometer method) serum protein fraction by means of electrophoresis on cellulose acetate plasma fibrinogen ad modum Bang (1) erythrocyte sedimentation rate in addition to whole blood viscosity Furthermore the conjunctival vessels of each subject were examined biomicroscopically for the degree of intravascular erythrocyte aggregation (7) The blood samples were drawn from an antecubital vein through a stainless steel needle no 18 with the aim tourniquet applied loosely Blood for viscosity measurements was collected in a plastic tube containing EDTA 10 mg per 10 ml of blood The sample was stored under slow rotation in a water bath at 37 C All viscosity measurements were

Table I *Hematocrit and shear stress at six different shear rates in patients with myocardial infarction and controls*

	No	Age (y)	Hematocrit ( )	Shear stress at shear rate (sec⁻¹) 230	115	46	23	11 5	5 75
Males									
1st day	20	59 4±9 5	45 7±4 3	11 67±1 52	6 56±0 84	3 17±0 40	2 02±0 28	1 3‸±0 33	0 94±0 18
21st day	11	55 5±7 2	41 6±4 1	9 93±1 10	5 57±0 34	2 68±0 30	1 72±0 22	1 14±0 16	0 81±0 14
Controls	15	60 7±4 6	45 5±2 9	10 53±1 28	5 86±0 75	2 87±0 40	1 78±0 29	1 11±0 22	0 74±0 17
Females									
1st day	5	61 4±7 3	44 8±5 4	11 06±1 99	6 22±1 08	3 01±0 55	1 91±0 39	1 22±0 25	0 86±0 18
21st day	4	65 5±7 7	38 5±2 2	8 92±0 92	4 93±0 54	2 36±0 31	1 47±0 25	0 92±0 16	0 58±0 16
Controls	15	66 5±7 8	42 3±2 2	10 02±0 8?	5 53±0 50	2 70±0 27	1 64±0 20	1 01±0 14	0 63±0 11

made within six hours at 37 C in a Wells Brookfield cone plate microviscometer (model LTV) This viscometer is capable of making determinations of shear stress at defined shear rates (10) Readings in the present study were taken in duplicate at six shear rates 5 75 11 5 23 46 115 and 230 inverse se onds (sec) The viscometer was calibrated with a silicone oil of 9 centipoise (cps) The reproducibility of whole blood viscosity readings was established from forty replicate readings on blood and the coefficients of variation were 3 01 2 84 2 04 1 79 1 79 1 67 and 1 65 per cent for increasing rates of shear between 5 75 and 230 sec

RESULTS

Since viscosity of a fluid is defined as the ratio of shear stress (dynes per sq cm) to shear rate (seconds⁻¹) viscosity = shear stress/shear rate the rheological characteristic of blood can be expressed directly in terms of the shear stress values at defined shear rates

In Table I the average values for hematocrit and for shear stress at six different shear rates are given for both sexes in patients with myo cardial infarction from whom blood samples were obtained on the first and on the twenty first day of the acute attack and in the controls

It is seen that the average hematocrit value are very much the same in cases of myocardial infarction on the first day as in the control sub jects (45 7±4 3 as compared to 45 5±2 9) *The whole blood viscosities are consistently higher in the patients with myocardial infarction than in the control subjects and these differences are sig nificant* ($p < 0\ 05$)

Parallel to these changes in the shear stress/ shear rate relationship Table II shows that al ready on the first day there are significant changes in the plasma proteins The total serum protein the alpha globulin the gamma globulin

Table II *Mean blood proteins ESR and degree of intravascular erythrocyte aggregation in patients with myo cardial infarction and controls*

	No	Blood proteins (g) Albumin	α_1 glob	α_2 glob	β glob	γ glob	Fibrinogen	Tot prot	Intra vascular aggre gation	ESR
Males										
1st day	20	4 57+0 52	0 ‸3±0 06	0 63±0 12	0 80±0 13	1 00±0 21	0 541±0 178	7 22±0 43	+ +	18±17
21st day	11	4 16±0 38	0 21±0 07	0 70±0 15	0 81±0 17	1 08±0 31	0 573±0 109	6 96±0 38	+ +	38±24
Controls	15	4 26+0 41	0 22±0 06	0 55±0 11	0 73±0 09	1 13±0 16	0 346±0 052	6 89±0 44	(+)	5±3
Females										
1st day	5	4 14+0 55	0 22±0 09	0 82±0 11	0 92±0 22	1 08±0 43	0 543±0 020	7 10±0 50	+	19±16
21st day	4	4 05±0 24	0 25±0 06	0 90±0 17	0 83±0 10	0 87±0 13	0 507±0 168	6 60±0 29	+ +	36±22
Controls	15	4 33±0 49	0 23±0 07	0 55±0 10	0 77±0 05	1 17±0 18	0 370±0 046	7 06±0 55	(+)	8±3

($p<0.05$) and particularly the plasma fibrinogen concentration are increased ($p<0.001$) in patients with myocardial infarction The average erythrocyte sedimentation rate is increased and there is an increased degree of intravascular erythrocyte aggregation

On the twenty first day of the infarction the average hematocrit value is significantly lower than on the first day (41 6±4 1 as compared to 45 7±4 3 $p<0.02$) and the whole blood viscosities are decreased ($p<0.05$) (Table I) The average plasma fibrinogen concentration remains significantly increased and the serum albumin concentration has significantly decreased from the first day of the infarction ($p<0.05$) the erythrocyte sedimentation rate is still higher than on the first day a is the degree of the intravascular erythrocyte aggregation (Table II)

COMMENT

Since blood behaves as a non Newtonian fluid the viscosity varies with the rate of shear and increases with decreasing shear rates The use of an Ostwald viscometer and other capillary tube viscometers do not permit quantitation of whole blood viscosity because the ratio of shear stress to shear rate cannot be quantified The use of the present cone plate viscometer with ethylenediamine tetraacetate (EDTA) as anticoagulant provides a precise in vitro method for whole blood viscosity measurements The shear rate range 230 to 5 75 sec^{-1} has been estimated to be within the shear rate range of blood flowing through the ascending aorta through medium sized ateroles to arterioles (11)

With this instrument the viscosity values of patients with myocardial infarction studied within 24 hours after the acute attack were found to be significantly different from those of the control group *This was not due to significant differences* in the hematocrit values This finding does not corroborate the result reported by Burch and De Pasquale of higher hematocrit values in patients with proved myocardial infarction The discrepancy between our finding and those of the latter investigators is difficult to evaluate It may be due to differences in patient material or in the time of obtaining the blood samples during this dynamic state of the disease The latter authors stated that the hematocrits of all patients were determined at the time of admission to the hospital but did not point out whether this was also the first day of the disease We made a very careful selection of patients with acute myocardial infarction uncomplicated by other illnesses and examined these patients within the first 24 hours of their disease By serial measurements of rheological components during acute myocardial infarction we as well as Langsjoen (9) observed a temporary increase in the hematocrit values on the second third and sometimes on the fourth day This finding as well as the observation of increased level of serum alpha globulin plasma fibrinogen increased degree of intravascular sludging progressive elevation of the erythrocyte sedimentation rate and decreased hematocrit and whole blood viscosity later during the course of myocardial infarction correlate well to the pattern of changes following acute tissue injury (2 8) It therefore appears that higher hematocrit and whole blood viscosity can not generally be considered as primary factors in the development of myocardial infarction but rather that an acute injury response is operative in acute infarction

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LACTATE DEHYDROGENASE OF HUMAN BONE MARROW IN THE STUDY OF HAEMOPOIESIS

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Abstract In two groups of patients one without haematological disease and one with various haemolytic anaemias total LDH activities and LDH isoenzyme patterns were determined in bone marrow plasma in extracts of nucleated cells from the bone marrow and in blood plasma

In haematologically normal persons LDH activity in marrow plasma was several times the activity found in blood plasma The isoenzyme distribution in marrow nucleated cells showed a predominance of isoenzyme 3 corresponding to the pattern of immature cells whereas marrow plasma showed a predominance of isoenzymes 2 and 3 Since this shift is towards the isoenzyme pattern of erythrocytes and away from the pattern of granulocytes it suggests that more enzyme is liberated from erythrocyte than from granulocyte precursors during cell maturation

In most of the haemolytic anaemias examined LDH activity in blood plasma was elevated The activity in marrow plasma did not differ from the control group This is taken as evidence that intramedullary haemolysis was not important in these states The increased erythropoiesis was reflected in the isoenzyme distribution which showed a shift towards the anodic isoenzymes

In the only case of myeloid hyperplasia examined the activity of the cathodic isoenzymes which are characteristic of granulocytes was so high as to suggest intramedullary destruction of granulocytic cells

Lactate dehydrogenase (LDH) exists in higher animals as five isoenzymes which can be separated by electrophoresis they are numbered according to their electrophoretic mobility no 1 being the most anodic isoenzyme The five isoenzymes are tetramers of two polypeptide subunits H and M and are thus composed HHHH HHHM etc The isoenzymes differ in functional properties and the carbohydrate metabolism of a tissue is in most cases reflected in its isoenzyme composition (12)

The isoenzyme patterns of the major cell types in human blood are well known and are summarized in Table I In normal human plasma isoenzymes 1 and 2 predominate (8) The sources of this activity have not been established but in conditions with tissue damage plasma LDH may rise the isoenzyme distribution reflecting that of the damaged organ Thus intravascular haemolysis is accompanied by a rise in the anodic isoenzymes in particular no 1 which is predominant in erythrocytes (18)

In the bone marrow LDH has been studied by Heller et al (7) who in four patients with blood loss anaemia found that the LDH activity in bone marrow plasma was twice the value found in blood plasma In one patient with megaloblastic anaemia due to folic acid deficiency it was shown that the pronounced elevation of LDH activity previously described in this condition was accompanied by a corresponding rise of activity in bone marrow plasma These authors suggested that the high plasma level of LDH in megaloblastic anaemia originated in the bone marrow and was due to increased release of enzyme from the megaloblastic cells In a later publication from the same laboratory (18) it was demonstrated that isoenzyme 1 was the most prominent in extracts of the buffy layer of megaloblastic bone marrow and a similar isoenzyme pattern was found in the serum of these patients

Elliott and Fleming (5) presented further evidence that the elevated plasma activity in folic acid deficiency was due to intramedullary destruction of erythrocyte precursors These workers and Spector et al (13) found that in haematologically normal patients LDH activity of bone marrow plasma was several times higher than the activity in blood plasma isoenzymes 2 3 4 were prominent

Table I *Lactate dehydrogenase isoenzyme patterns compiled from the literature of the main cell types in human blood*

Isoenzyme distribution

General description	Per cent H subunits[a]	Source
Erythrocytes		
Anodic pattern with predominance of isoenzymes 1 and 2	79	3 14
Granulocytes		
Cathodic pattern with predominance of isoenzymes 3 4 and 5	28	2 1
Lymphocytes		
Intermediate pattern with predominance of isoenzymes 2 and 3	66	Refs in 9
Thrombocytes		
Intermediate pattern with isoenzyme 3 as the most prominent	55	16 10

[a] Per cent H subunits is calculated from the contents of H subunits in the different isoenzymes

$$H = 100 \times \frac{[HHHH] + \frac{3}{4} \times [HHHM] + \frac{1}{2} \times [HHMM] + \frac{1}{4} \times [HMMM]}{[HHHH] + [HHHM] + [HHMM] + [HMMM] + [MMMM]}$$

Starkweather et al (15) found a predominance of isoenzyme 3 in normal bone marrow cells Their findings in leukaemic marrow suggested that blast cells display the usual intermediate pattern of immature cells with isoenzyme 3 predominating surprisingly (cf Table I) promyelocytes and myelocytes showed a predominance of isoenzymes 1 and 2 whereas the main isoenzymes of *nucleated erythrocyte precursors were 4 and 5* Dioguardi et al (4) also found an anodal isoenzyme pattern in immature myeloid cells from leukaemic patients granulocyte precursors from normal bone marrow however showed a predominance of isoenzymes 3 and 4

The present investigation was undertaken in order to test the hypothesis that the LDH activity of bone marrow plasma is a reflection of cell maturation and destruction during haemopoiesis A group of patients with haemolytic anaemias of various aetiologies was compared with a group of patients without haematological disease total LDH activities and LDH isoenzyme patterns were *determined* in bone marrow plasma in extracts of nucleated cells from the bone marrow and in blood plasma In addition one patient with myeloid hyperplasia was examined

MATERIAL AND METHODS

Thirteen patients with a normal haemoglobin concentration *and granulocyte count in the blood and a normal* bone marrow smear constitute the control material

Thirteen patients had haemolytic anaemias of various kinds paroxysmal nocturnal haemoglobinuria (PNH) (5 patients) microangiopathic haemolytic anaemia (2 patients) autoimmune haemolytic anaemia (2 patients) Waldenstrom s macroglobulinaemia (2 patients) acquired *idiopathic Coombs* negative haemolytic anaemia (2 patients) all these patients had a reticulocyte concentration above $50\,000/\mu l$ and the bone marrow smear showed erythroid hyperplasia

The patient with myeloid hyperplasia of the bone marrow suffered from regional enteritis His granulocyte concentration in the blood was normal but the blood smear showed a shift to the left including some metamyelocytes

Blood plasma was obtained using EDTA as anticoagulant Bone marrow was aspirated from the sternum (maximal volume aspirated was 1 ml) and collected in EDTA The marrow aspirate was transferred to capillary tubes and centrifuged at 3000 *g* for 20 minutes The aspirate was thereby separated into four sharply demarcated layers 1 fatty layer 2 marrow plasma 3 marrow buffy coat containing the nucleated cells and 4 erythrocytes *The cells of the buffy coat were* washed twice in Hanks solution and homogenized by grinding with a glass pestle the homogenate was centrifuged at 3000 *g* for 10 minutes and the sediment discarded

Total LDH activity was determined by the method of Laursen (11) (these analyses were performed by Dr Th Laursen) The samples were adjusted to approximately *the same LDH activity by dilution with Hanks solution* or concentration by vacuum dialysis LDH isoenzymes were separated by electrophoresis in agar gel according to Wieme (17) and visualized by van der Helm s tetrazolium method (8) The pattern was quantitated by scanning in a Vitatron spectrophotometer at 538 nm

Accidental haemolysis during bone marrow aspiration *will contribute to the LDH activity observed in marrow* plasma In order to evaluate the significance of this possible source of error erythrocytes were haemolysed mechanically The haemolysate was centrifuged at 3000 *g* for 20 minutes and corresponding values for total LDH activity and haemoglobin (6) in the supernatant were determined The results when compared to the maximal *haemoglobin concentrations observed in the plasma of* the aspirates indicate that enzyme leakage from erythrocytes maximally accounted for a few per cent of the LDH activity in bone marrow plasma

RESULTS

Thirteen patients without haematological abnormalities

Total LDH activity in blood plasma was 13–28 uM/h/ml In bone marrow plasma large variations were observed between samples probably in *the main due to varying degrees of admixture of*

blood. The highest value obtained in marrow plasma was 390 $uM/h/ml$ and in no case was the activity in marrow plasma less than four times the activity in blood plasma No correlation was found between the values in marrow plasma and in blood plasma

The isoenzyme pattern in blood plasma showed the well established predominance of anodic iso enzymes isoenzyme 1 or 2 being the strongest. In marrow plasma the pattern showed a more even distribution of subunits with isoenzyme 2 or 3 being the most prominent (Fig 1 *a*) The extracts of bone marrow nucleated cells showed predominance of isoenzyme 3

Thirteen patients with haemolytic anaemias and erythroid hyperplasia

Total LDH activity in blood plasma was elevated (more than 23 $\mu M/h/ml$) in nine of these patients. All the patients with intravascular haemolysis (PNH or microangiopathic haemolytic anaemia) had markedly elevated values (58–249 $\mu M/h/ml$) except one with only slight haemolysis (reticulocyte count 150 000/μl) who had borderline values for blood plasma LDH activity In bone marrow plasma LDH activity did not differ significantly from the values observed in the haematologically normal patients (highest value 369 $uM/h/ml$) There was no correlation between LDH activity in marrow plasma and number of reticulocytes in peripheral blood and no difference was observed between the different types of haemolytic anaemia examined

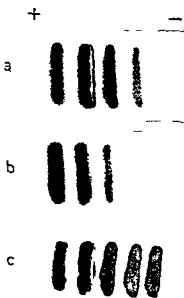


Fig 1 Lactate dehydrogenase isoenzyme patterns of bone marrow plasma (*a*) Normal (*b*) Erythroid hyperplasia (*c*) Myeloid hyperplasia

Table II *Lactate dehydrogenase activity in the plasma of bone marrow aspirates*

	Total LDH activity ($\mu M/h/ml$) Range	Mean	LDH isoenzyme distribution (cf Fig 1)
Controls 13 patients	70–390	191	Intermediate pattern with predominance of isoenzymes 2 and 3
Erythroid hyperplasia 13 patients	75–369	181	Anodic pattern with predominance of isoenzymes 1 and 2
Myeloid hyperplasia 1 patient	720		Cathodic pattern with predominance of isoenzymes 3 4 and 5

The elevated activity in blood plasma was due to an increase in isoenzymes 1 and 2 The marrow plasma of these patients when compared to the normal group showed a shift towards isoenzymes 1 and 2 (Fig 1 *b*) but contrary to what was seen in blood plasma isoenzymes 4 and 5 were clearly present The isoenzyme pattern in the nucleated cells of the marrow showed predominance of isoenzyme 2

One patient with myeloid hyperplasia

The total LDH activity and LDH isoenzyme pattern in blood plasma were normal In bone marrow plasma LDH activity was 720 $uM/h/ml$ the isoenzyme pattern showed a predominance of isoenzymes 3 4 and 5 (Fig 1 *c*) corresponding to the pattern seen in extracts of granulocytes

The results are summarized in Table II

DISCUSSION

In the plasma of bone marrow aspirates from haematologically normal patients LDH activities are several times the values observed in blood plasma This means that the unavoidable and varying admixture of normal peripheral blood during marrow aspiration will cause only minor modifications of the isoenzyme pattern Therefore the isoenzyme distribution in the aspirated plasma

probably reflects the pattern of the true interstitial fluid in the bone marrow Since this is markedly different from the pattern in blood plasma it appears unlikely that the bone marrow is the main source of the LDH activity in blood plasma

Usually elevated extracellular LDH values are considered indicators of cellular damage with subsequent loss of enzymes from the cell Interpreted in this way the high LDH values in marrow plasma would be evidence of loss of cells during haemopoiesis i e normal haemopoiesis would involve some degree of ineffective haemopoiesis This would affect mainly the late erythroid precursors since the isoenzyme distribution in marrow plasma when compared to the pattern of marrow nucleated cells shows a shift towards the anodal pattern characteristic of erythrocytes (Fig 1 *b* vs Fig 1 *a*) further evidence of this cell loss during erythroid development is the early appearing bilirubin demonstrable after the administration of labelled glycine (19) Another possible explanation of the findings is a loss of cytoplasmic enzyme during the expulsion of nuclei from normoblasts Finally destruction of aged red cells in the bone marrow cannot be excluded as a contributory factor however the fact that none of the patients with haemolytic anaemia showed higher LDH activity in bone marrow plasma than normal persons argues against this explanation

Studies on megaloblastic anaemias (5 7 18) have shown a marked elevation of LDH activity in bone marrow plasma indicating a considerable degree of intramedullary haemolysis In the present study several types of haemolytic anaemia were examined in none did marrow plasma LDH activity exceed the values seen in the control group This suggests that in these haemolytic anaemias intramedullary destruction of cells is no more prominent than in normal persons The shift towards the anodic isoenzymes observed both in bone marrow nucleated cells and in bone marrow plasma merely reflects the increased erythropoiesis

The highest LDH activity of bone marrow plasma was found in a patient with myeloid hyperplasia the activity was comparable to that observed by others in megaloblastic anaemia The isoenzyme distribution showed predominance of the cathodic isoenzymes which are prominent in granulocytes (Fig 1 *c*) This means that the activity of these isoenzymes was increased so enormously in comparison with normal bone marrow plasma that the most likely explanation is intramedullary destruction of late myeloid precursors or of granulocytes

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ELEVATED BODY TEMPERATURE AND THE SURVIVAL OF RED BLOOD CELLS

A Study on Experimental Pyrexia in Rabbits

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Abstract When elevating the body temperature of rabbits, whose red cells were labelled with Cr of DF³²P by injecting bacterial pyrogen injecting heated milk or by external heating in a climatic chamber it was found that most probably pyrexia entails increased haemolysis

Comparative animal investigations have indicated a correlation between metabolism and the erythrocyte turnover (20) A particularly long red cell life span has been found in poikilothermic animals (7) Moreover the survival time of the red cells is prolonged during hibernation (5 17) On the other hand there do not seem to have been any studies on erythrokinetics during variations in body temperature within the usual biological temperature interval especially during fever although widely different clinical conditions entailing elevated body temperature may be associated with anaemias having a haemolytic component

Few authors have been aware of an association between haemolysis and elevation of temperature In Hodgkin s disease haemolytic crises have occurred synchronously with Pel Ebstein fever (19) experimental pyrexia gave a decrease in the ^{51}Cr survival time in sickle-cell anaemia (3) and increased haemolysis has been observed on heat exposure of mice with spherocytosis (1)

On this basis the author performed studies on the survival of red cells in rabbits during experimental elevation of body temperature by 2-3 C

MATERIAL AND METHODS

Rabbits of both sexes wei hing 500-4000 g were used I v injections were g en into and blood samples drawn from the marginal ve n of the ear

The red cells were labelled with Cr as Na_2 CrO from The Radiochemical Centre Amersham The specific activity on delivery was 50-150 mCi/mg Cr In two cases Na CrO from Kjeller Norway specific activity 280 mCi/mg Cr was used The dose used for labell ng was 25-50 μCi never exceeding 1 μg Cr per ml red cells In the labelling procedure about 4 ml blood was used to this was added 1 ml of a glucose-citrate solution After centrifuging at 1700 rpm for 10 min plasma was p petted off and mixed with 10 ml NaCl 0 9 for subsequent washing The erythrocyte suspension was incubated with Na CrO for 45 min at room temperature The red cells were washed thrice with 0 9 NaCl solution resuspended in the same solution, and a known amount was reinjected The first blood sample was drawn 15-30 min later and further samples were drawn every 2 to 3 days for 35-40 days. Measurement of radioactivity was carried out on 500-1000 μl whole blood in a well scintillation counter with a one-channel analyser At least 4000 counts were measured

Diisopropyl fluoro³²phosphate (DF³²P) labelling was done with DF P from the Rad ochemical Centre Amersham Specif c acti ity on delivery 200-400 μCi/mg DFP Before use the preparation was diluted with propylene glycol The labelling was done in vivo by an i v dose of 40-50 μg DFP corresponding to 4-20 μCi P Blood samples of approx mately 1 ml were drawn into heparinized tubes the f rst sample about 1 hour after the injection and then at varying intervals for up to 45 days With a Carlsberg pipette 1 0 ml was emo ed centr fuged at 1700 rpm for 10 min and the plasma was p petted off Thereafter the sample was washed thrice w th NaCl 0 9 solut on and the f nal volume was brought to 1 3 ml 1000 μl of this final suspension on which haematocrit dete mination was done was placed with a few drops of sapon n solution (5) and 200 μl 50 sucrose solution as vehicle in a planchette After drying at room temperature the sample looks l ke a smooth arnish like film The act ity wa measured with G M tubes ha ing a window of 1 7 mg sq cm At least 4000 counts were measu ed No orrection was made for self absorpt on as ontrol st dies showed that the flu tuations n the haematocrit value were insignif cant

The act vity is stated partly as count per ml whole

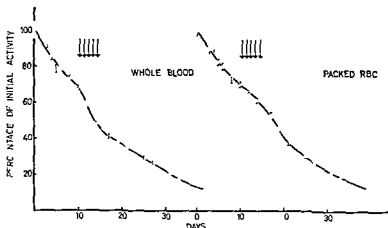


Fig 1 The influence of five days with fever produced by i v injection of bacterial pyrogen (*arrows*) on the survival of ^{51}Cr labelled erythrocytes in a rabbit Activity expressed as counts per ml blood (*left*) and counts per ml red blood cells (*right*) in percentage of value at time zero

blood and partly in relation to the haematocrit or haemoglobin value In ascertaining survival curves no regard was paid to loss of blood during the sampling The coefficient of variation in the Cr method was 2 5 and in the DF^{32}-P method 3

The haemoglobin concentration was determined spectrophotometrically as oxyhaemoglobin after dilution with dilute ammonium hydroxide The number of reticulocytes was counted after staining with brilliant cresyl blue

An elevation of the body temperature was induced by three methods

1 Intravenous injection of bacterial pyrogen (vaccine for fever therapy (made in Statens Seruminstitut Copenhagen) containing pyrogen corresponding to 100 mill bacteria per ml of a microorganism of the *Alcaligenes faecalis* group) The vaccine was administered in daily doses of 0 75–2 5 ml

2 I m injections of heat treated cow s milk Apyrogenic utensils were used for heating the milk (100 C for 15 min) and for the injection The dosage was generally 5 ml

3 External heating in a hyperthermia apparatus consisting of a double walled chamber whose temperature is regulated in connection with a thermostat mechanism insufflation of heated air into the space between the walls

The rectal temperature was recorded in some of the experiments by a thermistor encased in a 6 cm long polyethylene cover the end of which was inserted about 7 cm into the rectum The reading was done continuously with a graphic recorder In addition a recording apparatus according to the thermocouple principle was used for the simultaneous recording of the temperature on 10 rabbits (Electro Universal Thermometer Ellab Instruments Copenhagen lent us by the Control Laboratories of the Danish Pharmacist Association) The sensitive point placed at the tip of the rubber tubing was inserted about 7 cm into the rectum

The temperature response after injection of pyrogen appears after a latent period averaging 1–1 ½ hours and the curve describes a typical biphasic course The maximum increase was 1–3 C above the rabbit s normal rectal temperature of 38 5–39 0 C and the elevation subsided within 10–12 hours The findings were somewhat different after the injection of milk which gave only one maximum peak with a more pronounced plateau In the hyperthermia method the rectal temperature was kept between 41 and 41 8 C for 6 8 hours a day

In principle the following procedure was used in studying the breakdown of erythrocytes during pyrexia During an initial phase of 8 10 days the spontaneous course of the red cell survival curve was studied Then followed a period of induced fever with daily elevations of temperature as described above Blood samples for assessing alterations in the red cell activity were always taken just before induced elevation of the temperature i e more than 10 hours after the previous attack of fever had subsided

RESULTS

Injections of bacterial pyrogen into rabbits with ^{51}Cr labelled red cells

Fig 1 shows the curve representing the disappearance of ^{51}Cr from the circulation in a rabbit in connection with a 5 day period of fever induced by daily injections of bacterial pyrogen beginning 10 days after the autologous red cells had been labelled The curve on the left shows the counts per ml whole blood During the induced fever there is a temporary steeper fall whereupon the curve again flattens At the same time there was also a fall in the haematocrit and haemoglobin values When the activity per ml red cells or per g haemoglobin is depicted the steep decrease in the curve is shifted towards the right as seen from the right part of the figure During the fever the curve appears as a continuation of the spontaneous course A fall does not occur until after the febrile period or during the latter part of the febrile period The experiment was carried out using varying amounts of pyrogen on a total of 25 rabbits In 18 there was

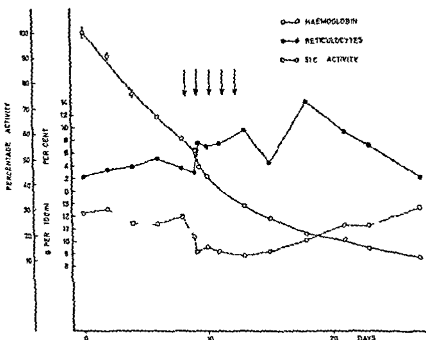


Fig 2 The influence of five days with fever produced by i v injection of bacterial pyrogen (arrows) on the survival of ^{51}Cr labelled erythrocytes haemoglobin and reticulocyte values in a rabbit Activity expressed as counts per ml blood in percentage of value at time zero

a distinct change in the level of the curve of the type just described Fig 2 gives the result of a similar experiment including the changes in haemoglobin and reticulocytes in relation to fever

Injections of bacterial pyrogen into rabbits with DF^{32}P labelled red cells

The experiment was repeated after labelling the red cells with DF^{32}P and the result shows the same pattern (Fig 3) This experiment was carried out on eight rabbits seven of which showed the named change with quantitative findings in the same range as that seen with the ^{51}Cr method

In vitro investigation of the direct effect of bacterial pyrogens upon rabbit red cells

Rabbit blood was incubated with bacterial pyrogen in four times the expected initial concentration in the blood stream in the in vivo experiments The experiment was carried out at 37 and at 41 5 C and the effect was assessed by determining either free haemoglobin or the ^{51}Cr activity in the plasma by removing samples at varying intervals for up to 24 hours and comparison with blood from the same rabbit without addition of pyrogen In no case there was any difference in the autohaemolysis determined in this way

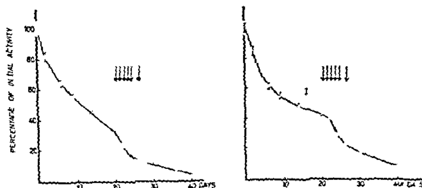


Fig 3 The influence of six days with fever produced by i v injection of bacterial pyrogen (arrows) on the survival of DF^{32}P labelled erythrocytes in two rabbits Activity expressed as counts per ml packed red blood cells in percentage of value at time zero

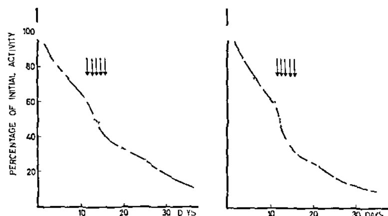


Fig 4 The influence of five days with fever produced by i v injection of heat treated milk on the survival of Cr labelled erythrocytes in two rabbit Activity expressed as counts per ml blood in percentage of value at time zero

Injections of heat treated milk into rabbits with ¹Cr labelled red cells

The effect of an elevation of tempe ature indu ed by daily injections of heated milk was studied on six rabbits after labelling with ¹Cr In four out of six cases a 5–6 day period of fever caused a change in the red cell survival curve as de scribed Two such experiments are seen in Fig 4

Rabbits with ⁵¹Cr or DF³ P labelled red cells studied during hyperthermia in a climatic chamber

An elevation of the temperature was brought about as described The rabbits were placed daily in the climatic chamber and the rectal tempera ture recorded continuously was kept between 41 and 41 8 C for 6–8 hours daily Labelling both with ¹Cr or DF³ P was used Fig 5 illustrates the red cell survival curve of two ⁵¹Cr labelled rabbits As in the other methods of inducing fever there is a more rapid fall of the curve while the body temperature is elevated Experi ments were done on five ⁵¹Cr labelled and one DF³ P labelled (Fig 6) rabbits Four showed an evident fall one a doubtful and one no change

Reticulocyte counts

The fall in haematocrit observed during the feb rile period returned to normal simultaneously with an accentuated fall in counts per ml red cells or per g haemoglobin These findings could be due to a release of unmarked erythrocytes into the circulation which could be demonstrated by an increase in the reticulocyte count as shown in Fig 2 Table I gives a comparison of the mean reticulocyte values in 12 rabbits before during and after the period of fever produced by bac terial pyrogen there was a significant increase in the reticulocyte counts as the other haematological parameters returned to normal A similar retic ulocytosis was also seen in studie with external heating

DISCUSSION

Deviations in the survival curve per volume unit of whole blood of tracer labelled red cells as

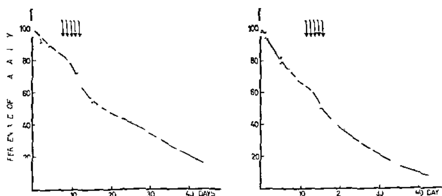


Fig 5 The influences of hyperthermia treatment for five days (each arrow re presenting treatment for 6 8 hours) on the survival of Cr labelled erythrocytes in two rabbits Activity ex pressed as counts per ml blood in percentage of value at time zero

found in the present study are not erythrokinetically consistent and cannot alone be taken to represent a haemolytic state A similar decrease would also occur in haemodilution which could be present in fever (2) When expressing the activity as counts per unit of packed red cells or per g haemoglobin there is better evidence of recent haemolysis The deviation in this curve is best explained by the release of new unlabelled cells and this is confirmed by the increase in the reticulocyte counts By using a double isotope technique evidence of a variation in temperature sensibility of the red blood cells with cell age could be obtained (14) a finding which speaks against the haemodilution explanation The identical curve patterns and uniform quantitative changes with ⁵¹Cr and DF³²P labelling militate against a temperature-conditioned loss of marker activity in particular an increase of chromium elution from intact cells

It could be visualized that the effect of the bacterial pyrogen was due to a direct toxic action of the red cells considering that bacterial endotoxin of the lipopolysaccharide type can be bound to red cells in the haemagglutination test (18) In vitro incubation with large quantities of the pyrogen used in the present study did not entail any haemolysis Pyrogen has been shown to have no effect upon the viability of red cells during storing of blood (10) but is believed to have a detrimental effect upon the red cells only in the presence of specific antibodies directed against the endotoxin (18) Ho and Kass (12) and Shumsway et al (22) have found signs of a pyrogen induced haemolysis under circumstances in which immune processes were believed to be operative Keiderling (15) demonstrated an altered ⁵¹Cr half life in rabbits subjected for a long period to injection of various pyrogens The temperature findings are not mentioned in these experiments which were supposed to imitate a state of infection Immunological factors can hardly be imagined to have been operative in the present experiments which immediately caused a loss of red cells Recently Brain and Hourihane (6) have induced haemolysis by injecting coli lipopolysaccharide into rabbits The finding was interpreted as microangiopathic haemolytic anaemia in association with a generalized Shwartzman reaction which often proved fatal In the present experiments the rabbits were always clinically unaffected by the pyrogen injections Gross changes of the organs were never seen and smears ex

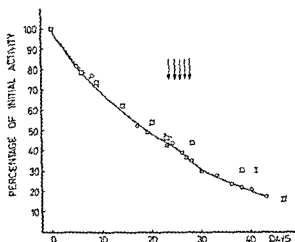


Fig 6 The influence of hyperthermia treatment on the survival of DF³²P labelled erythrocytes in a rabbit Activity expressed as counts per ml red blood cells in per cent age of value at time zero Two examinations in the same animal I without treatment II four months later with hypertherm a treatment for five days (each arrow representing treatment for 6–8 hours)

Table I *Reticulocyte and haematocrit values in 12 rabbits in relation to febrile period produced by bacterial pyrogen*

	No of trials	Mean haematocrit value	Mean reticulocyte value	*T* test
Prefebrile period	19	36 4 ± 3 8	29 4 ± 11 5	
Febrile period	12	34 5 ± 4 5	33 5 ± 14 0	$p > 0\,30$
First postfebrile period (during accentuated slope in cpm ml RBC)	35	31 6 ± 4 6	65 8 ± 28 6	$p < 0\,001$
Second postfebrile period	12	33 0 ± 4 6	29 5 ± 6 9	

hibited no definite morphological changes of the red cells

Elevation of body temperature induced by injecting heat treated milk entailed the same changes in the red cell survival pattern as did bacterial pyrogen The reason for the fever is not known with certainty Fat emulsions may entail febrile reactions (16) Contamination with pyrogen has also been advanced as a possible explanation (21) but to this it may be objected that the temperature curve in the present experiments was of an appearance differing from that of the pyrogen studies lacking the typical biphasic course The experiments utilizing external heating in a climatic chamber are more conclusive in respect to the significance of temperature for the degree of haemolysis It was the elevated body temperature which was the common factor in these experiments which had such a uniform effect

For the present study no attempt was made at a quantitative assessment of the extraordinary loss A model must be set up permitting a correct estimate of the magnitude of the loss and thereby of a possible relationship between the increased temperature and the degree of the destruction

The present study does not permit conclusions concerning the mechanism of the accelerating influence of fever upon the destruction of red cells One might imagine an action direct upon the metabolism of the red cells a damaging effect upon the erythrocyte membrane or a functional change in cellular systems influencing the destruction of red cells primarily the reticuloendothelial system (R E S) Bacterial pyrogens are known to stimulate the phagocytic activity in the R E S (4) and an activating effect upon the R E S is also believed to be to some extent the background of the effect of non specific thermotherapy including hyperthermia (13 24)

High temperatures may destroy the red blood cells in vitro and in vivo This is known from the anaemia following thermal injury (8) and haemolysis has been described in heat stroke (9) The anaemia following thermal injury has been further elucidated in vitro (11) It has been demonstrated that the same change in red cells e g assessed by a reduction in osmotic resistance may be induced either by a considerable elevation of temperature or by extending the duration of the action Since investigations of this nature have been concerned with imitating the burn situation they have been restricted to actions of up to one hour and have shown no changes until temperatures of 46 C and higher has been used In determining the ^{51}Cr survival time after in vitro action of heat the criterial limit at 20 min has been found to be 46–48 C (23) On this basis it is not *unreasonable to imagine that an extension of the* exposure time as in the present study may shift the critical temperature to the biological range of pyrexia Further investigations of the mechanism are in progress

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